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# EFFECTS OF TEMPERATURE ON GROWTH, ANATOMY, AND METABOLISM OF APPLE AND PEACH ROOTS[1]

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(WITH ELEVEN FIGURES)

## Introduction

Several publications have recently appeared in which effects of temperature were emphasized in their relation to the growth and metabolism of the aerial organs of plants (**18, 22, 23**). The studies here recorded are concerned principally with influences of temperature on the growth, anatomy, and metabolism of the roots of apple and peach. The results were obtained through simultaneous studies of progressive changes in composition and anatomy as they occurred in the growth of the roots concerned at the respective temperatures employed.

The value of anatomy in the interpretation of macrochemical analysis and, conversely, of chemistry in interpreting anatomical responses, was repeatedly demonstrated. Much apparent aberrancy in the interpretation of the results of macroanalysis of plant organs may well be due to lack of knowledge of the anatomical development of the material analyzed.

[1] Through the courtesy of the University of Chicago there was made available for this work the temperature control equipment and laboratory facilities of its department of botany. The writer wishes particularly to acknowledge the advice and generous cooperation of E. J. KRAUS and M. A. BLAKE.

Journal series paper of the New Jersey Agricultural Experiment Station.

## Experimental methods

### APPLE TREES

The apple trees used in these experiments were root grafts of the Stayman variety, and when received from a commercial nursery were 45–50 cm. in height. About 400 trees were selected for uniformity from a much larger population, and on December 1, 1933, after the dormant trees had been several weeks in a cold storage cellar, they were washed free of soil and all the fine fibrous roots removed so that there remained only old roots, none of which was less than 1 cm. in smallest diameter. The tops were also cut back so that when planted they were 30 cm. in height. This left several buds.

TABLE I

COMPOSITION OF NUTRIENT SOLUTION
PARTIAL VOLUME MOLECULAR CONCENTRATION OF SALTS USED

| | $Ca(NO_3)_2$ | $KH_2PO_4$ | $MgSO_4$ | $CaCl_2$ |
|---|---|---|---|---|
| Complete or plus-$NO_3$.. | 0.0090 | 0.0045 | 0.0045 | ..... |
| Minus-N..... | ..... | 0.0045 | 0.0045 | 0.0090 |

Only one of these, the most distal bud, was allowed to remain; otherwise the number of growing points per tree would have been variable, thereby making more difficult the determinations of amount and quality of growth.

The trees were then set four to a 12-liter, self-draining porcelain jar. Some coarse glass wool was placed over the drainage outlet in the center of the bowl-shaped bottoms and the jars were nearly filled with nitrogen-free white quartz sand, which had been sifted so that all particles passed through a 1.0 mm. sieve and were held by a 0.5 mm. sieve.

Until January 31, 1934, the trees remained in the culture jars in a storage room at 35°–38° F. (all temperatures herein mentioned are Fahrenheit) and received an application of minus-N solution (table I) once a week in sufficient quantity to flush the sand. The cultures were kept constantly moist by the application of tap water as required. At that time, 30 trees (hereafter referred to as initial trees)

were harvested for chemical analysis. The remainder of the trees in the porcelain jars were shifted to water baths, as described by LINK,[2] which were thermostatically regulated to $\pm 0.2°$ to give the respective sand temperatures indicated in table II. At each tempera-

TABLE II

ELBERTA PEACH TREES
AVERAGE CURRENT GROWTH PER TREE AT DIFFERENT SAND TEMPERATURES

| Sand temperature (°F.) | Fresh weight in grams | | | | | | Length in centimeters | | |
|---|---|---|---|---|---|---|---|---|---|
| | Roots | | | Stem and leaves | | | Stem | | |
| | Mar. 17 | April 4 | | Mar. 17 | April 4 | | Mar. 17 | April 4 | |
| | −N | −N | $+NO_3$ | −N | −N | $+NO_3$ | −N | −N | $+NO_3$ |
| 45 | None | None | 0.2 | 0.7 | 2.0 | 2.0+ | 0 | 0 | 0 |
| 50 | 0.3 | 4.5 | 5.0 | 1.5 | 3.0 | 9.0 | 5 | 7 | 12 |
| 55 | 7.0 | 7.0 | 7.0 | 5.5 | 6.0 | 14.0 | 12 | 25 | 28 |
| 60 | 8.5 | 11.0 | 13.0 | 7.5 | 7.5 | 14.5 | 20 | 25 | 33 |
| 65 | 8.0 | 12.0 | 15.0 | 8.6 | 11.5 | 21.0 | 23 | 30 | 41† |
| 75 | 5.0 | 7.0 | 6.0 | 4.7 | 8.0 | 19.0 | 18 | 23 | 38 |
| 85 | 2.0 | 2.2 | 2.0 | 3.5 | 6.0 | 7.2 | 12 | 18 | 25 |
| 90 | 0.4 | 0.2 | 0.2− | 3.2 | 3.5 | 5.0 | 7 | 10 | 15 |
| 95 | None | ..... | ..... | 0.7 | ..... | ..... | 5 | ..... | ..... |
| 60–45* | 8.5 | 9.2 | 9.8 | 7.5 | 8.6 | 9.0 | 20 | 23 | 25 |
| 60–95* | 8.5 | 7.0 | 6.0 | 8.5 | 8.8 | 9.0 | 20 | 33 | 38 |
| 90–60* | 0.4 | 2.0 | 1.5 | 3.2 | 7.0 | 5.0 | 7 | 12 | 12 |
| 45–60* | None | 8.9 | 9.5 | 0.7 | 5.0 | 7.2 | 0 | 10 | 15 |

* Shifted March 17.

† In addition to 41 cm. of primary current stem there was lateral stem growth which alone was 44 cm. in length.

ture there were employed 36 trees. The cultures were each provided with independent drainage by means of a tube with watertight connections which ran with a downward slope from the outlet in the bottom of each jar out through the side walls of the temperature bath. Here the drip from the cultures was frequently collected for determinations of pH values. The temperature of the air in the greenhouse was in all cases held at approximately 60°–65° at night and 65°–70° during the day.

In the following discussion all references to temperature, unless

[2] LINK, G. K. K., Science **81**:204-207. 1935.

otherwise indicated, will refer to the temperature of the sand in the culture jars.

With a few exceptions, to be mentioned elsewhere, all of the apple trees received daily applications of the complete or plus-$NO_3$ solution (table I) in sufficient quantity to flush the sand, and once a week each culture was washed with several liters of distilled water after which nutrient solution was immediately applied. Particularly at the higher temperatures, the sand in the culture jars dried out to a depth of 1 or 2 cm. This dry surface layer apparently acted as a mulch, however, as even at 95° there was always an abundance of water in the sand at lower levels.

### Peach trees

About 400 uniform "one-year-old" Elberta peach trees 90–100 cm. in height were also used in these experiments. They were pruned back to a height of 30 cm. and otherwise handled in the same manner as the apple trees, except as to nutrient treatment. When shifted from cold storage to the temperature baths on January 31, all of the peach trees continued to receive the minus-N solution until March 17. On that date and until finally harvested, some of the peach trees were supplied with the complete or plus-$NO_3$ solution and others continued to receive the solution lacking nitrogen (table I). The amount and frequency of application of solution, however, were exactly the same as already described for the apple trees. Ferrous sulphate was applied to the peach trees to avoid iron deficiency (**13**). This was not necessary in case of apple. Boron and manganese were present in sufficient amount either in the salts applied or in the tissues of the initial trees of both peach and apple.

On March 17, some of the cultures of both apple and peach were shifted from one sand temperature to another, as indicated in table II.

Methods of chemical analysis have been described in recent publications (**4, 22**). The current fibrous roots from ten or more trees were removed and analyzed macrochemically as a single sample. The old large lateral roots from the same trees were employed as a second analytical sample. They varied from 0.5 to 1.5 cm. in

diameter and were from 8 to 10 cm. in length (figs. 1–3). Macro-analyses of other parts of the trees are not reported in detail.

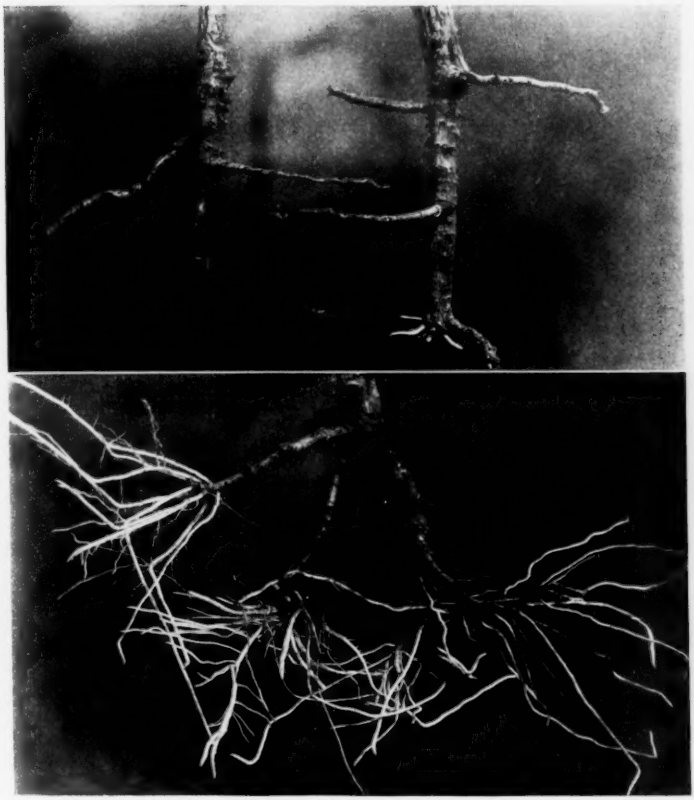

FIG. 1.—Peach roots March 17. Upper left, 45°; upper right, 50°; lower illustration, 55° F. Note that there are no new roots at 45°, few at 50°, and at 55° many rather short, white, typically large-diameter succulent roots with few fine laterals.

ECKERSON'S (6) method was employed for reducase determinations, except that instead of adding 9 cc. of distilled water to 1 cc. of standard nitrite solution there was added 9 cc. of nitrite-free extract from an aliquot of the tissue to be tested for reducase activity.

The extracts were invariably yellowish brown in color and this method of preparing the standard solution facilitated the making of color comparisons.

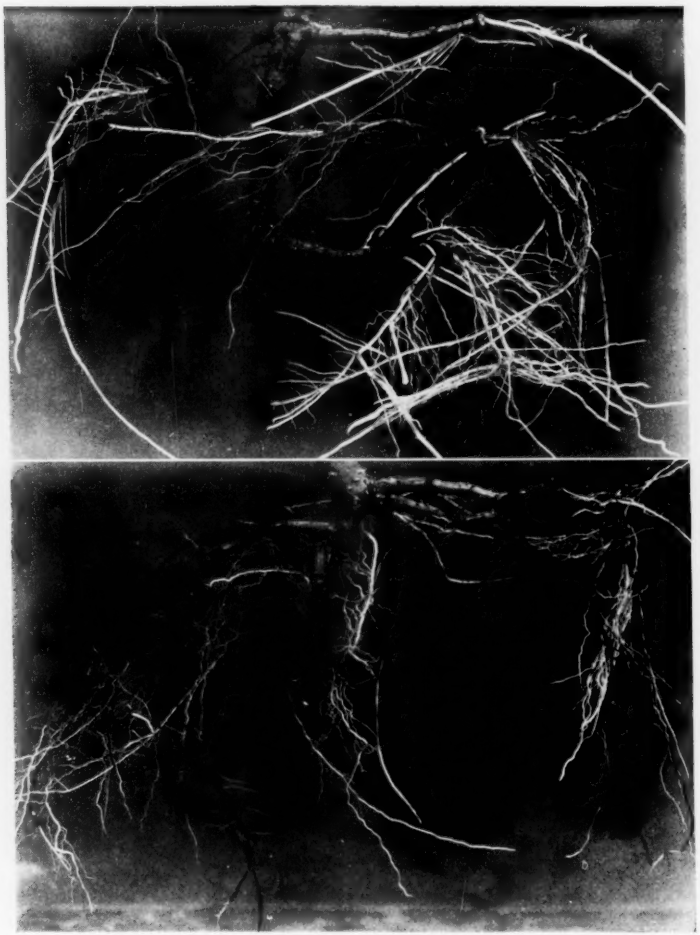

FIG. 2.—Peach roots March 17. Upper illustration, 65°: current roots more extensive but similar in quality to those at 55°; lower illustration, 75°: note dead cortex and fine laterals. These roots lacked succulence.

The usual microchemical methods (9) were followed and the reagents and range indicator method described by SMALL (27) were used in determining the pH values of various plant tissues.

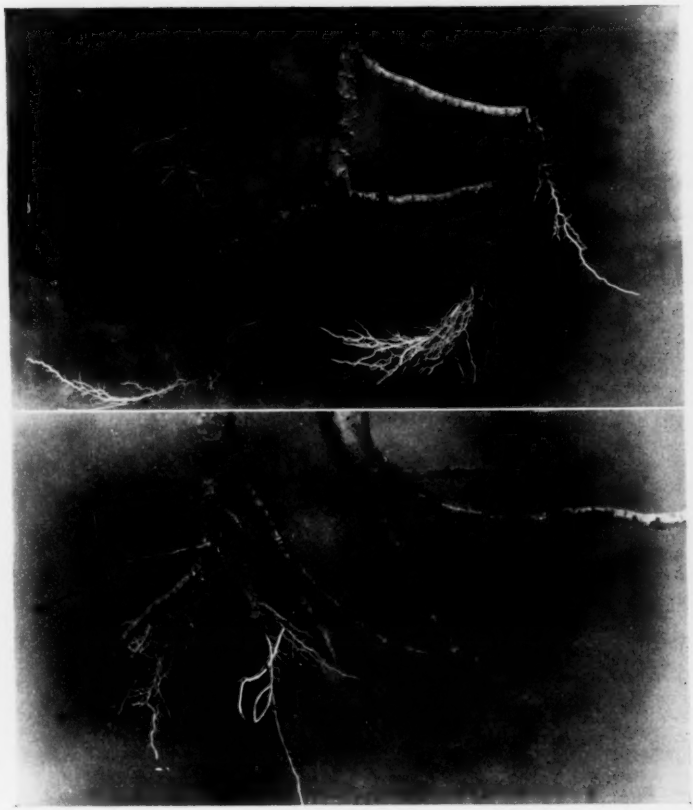

FIG. 3.—Peach roots March 17. Upper illustration, 85°: limited development of non-succulent current roots of very small diameter with short-lived cortex and numerous fine laterals; lower left, 90°: roots much like those at 85° but less extensive; lower right, 95°: no new roots developed.

In preparing permanent slides, already well recognized methods were employed that need not be described in detail. Material was fixed in Navashin's solution, dehydrated, imbedded in paraffin, sec-

tioned, and stained with safranin, gentian violet, and gold orange. All drawings were made on the same scale with the aid of a projectoscope.

The pH estimations of nutrient solutions were made colorimetrically using as standards the colored glass discs of a Hellige Klett color comparator. Solutions were tested before and after passing through the sand of the cultures, necessary adjustments being made with 0.1N KOH or $H_2SO_4$ as required. The solutions as applied were approximately pH 4.5 but after passing through the sand they frequently reached a pH value of 5.8–5.5. These conditions are such, however, as to permit good growth and fairly vigorous assimilation of nitrate (**3, 19, 31, 32**) if other environmental factors are favorable.

## Discussion of results

### External appearance of apple and peach roots at different temperatures

When the initial apple and peach trees were shifted from cold storage to the respective temperature baths on January 31, they had no fibrous roots, no callus tissue had developed on the pruning wounds of the large roots, and the buds had not enlarged noticeably. The trees were therefore comparatively inactive when experimental treatments were commenced. The results reported here are consequently in contrast to those recently (**19, 22, 23**) secured with actively growing fruit trees which were shifted to various extremes of temperature when they had initially present a well developed system of current fibrous roots and tops.

On March 17, after the cultures had been for 45 days at the respective sand temperatures indicated in table II, photographs were taken of typical root systems of peach (figs. 1–3) and average weights were recorded for both peach (table II) and apple (fig. 11). It is apparent that in both genera the greatest amount of roots was produced at 65°. In both cases the current roots weighed slightly less at 60° and 75° and still less at the greater extremes of high and low temperature. The minimum soil temperature at which roots will grow undoubtedly varies, however, not only with the genus but with the variety (**15, 24**). Rogers (**25**) reports that in England actively

growing roots were found on pear, gooseberry, and black currant in February, 1930, while the tops were still dormant. It is likewise recorded in a recent report (**19**) that the initially present fibrous roots of Delicious apple trees increased materially in volume at 48° although there was practically no growth of tops at that temperature. GOFF (**11**) found that the root growth of many perennial plants started considerably in advance of top growth.

At 45° a few new fibrous roots appeared on the apple trees, all of which it will be recalled received the complete or plus-$NO_3$ nutrient solution (table I). A similar response occurred in case of all the peach trees with plus-$NO_3$ treatment at 45°, but none of the peach trees at this temperature which lacked an external nitrogen supply formed any new roots during the period of these experiments. This point may be of considerable practical value and will be discussed elsewhere in connection with reducase activity and effects on peach of added nitrate at the other sand temperatures employed.

When the peach trees at 60° with a well developed system of new roots were shifted to 45°, however, there was some growth of fibrous roots in case of both nutrient treatments (table II). The same situation also prevailed in case of apple when shifted from 60° to 45°.

At 95°, the highest temperature employed, there were no new roots produced in either genera. It has been shown (**22, 23**), however, that the initially present current fibrous roots of various varieties of apple and peach survived this temperature for a period of several days, although the roots were injured internally more or less seriously, depending upon the variety. As in the previous case, the peach and apple trees did not lose their fibrous root system, although the cortex died almost at once when shifted from 60° to 95° for a period of 18 days. Also the roots in a few days exhibited internally other characteristics which, as will be shown later, are typical of high soil temperatures.

Before leaving the subject of gross weight or volume of apple and peach roots produced, particular attention is called to the effects of comparatively small differences in temperature. At 85°, for example, the root systems of both genera were extremely small, yet at 75° there was in both cases a large volume of roots. Note also the amount of roots produced respectively by apple and peach at 50°

as compared with 55°, and the roots produced at the latter temperature in relation to those developed where the sand was maintained at 65° (figs. 1–3, table II). It would seem apparent that the amount of root growth can be greatly modified by only a very few degrees' difference in the temperature of the soil.

Although the amount of root growth at different soil temperatures is important, at least of equal significance is the character or quality of roots produced. Note the peach roots produced at temperatures below 75° as shown in figures 1 and 2 on March 17. (And at comparable temperatures the apple roots were essentially the same, except that they started root growth a little later.) The roots were glistening white and typically of relatively large diameter. The cortex was still alive, as indicated by the fact that practically none of the roots were brown, even at their older or proximal end. A further characteristic of these roots was their extreme succulence; a comparatively slight bending caused them to break completely in two. On the other hand, at 75° and higher the current roots lacked succulence, except near the tips. The extreme toughness of these recently developed but non-succulent roots is evident from the fact that they could be tied in a knot without breaking. Figures 2 and 3 show also that even on March 17 much of the cortical tissue was brown and dead at 75° and higher.

That the current top growth of both apple and peach was closely correlated with the volume of roots produced, is shown by figure 4 and by the weight and linear growth of tops as recorded in table II. At the sand temperatures 45°, 50°, 90°, and 95°, the leaves were somewhat curled (fig. 4), undoubtedly owing to inability of the trees to absorb an adequate amount of water in the practical absence for several weeks of a fibrous root system.

In case of the peach and apple trees which on March 17 were shifted from 60° to 45° and to 95° respectively, there was present of course a well developed fibrous root system. These trees apparently absorbed water freely at 45° and 95°, as indicated by the fact that the tops of the trees of neither genus showed any sign of curling or wilting. The extremes of temperature evidently permitted intake of water.

As already mentioned, however, no fibrous roots developed and

none were present on any of the fruit trees continually at 95°, nor on those lacking nitrate and continuously at 45°. Nevertheless the tops of the trees did not die nor did the current top growth wither,

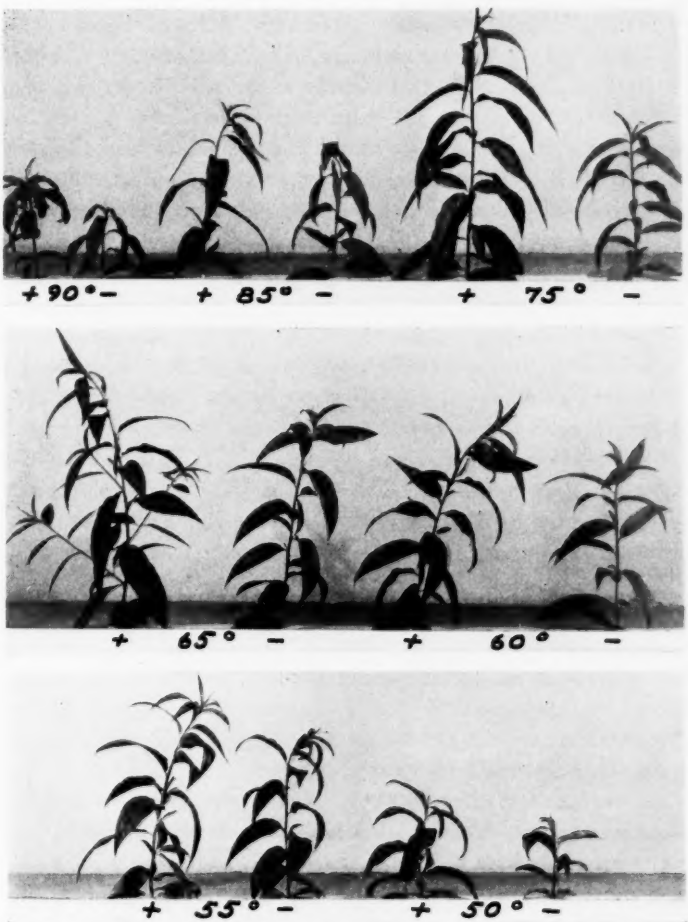


FIG. 4.—Current top growth of peach April 4. All tops grown at about 65°; roots at temperatures indicated. The plus-$NO_3$ solution is designated by the plus sign and the minus-N solution by the minus sign. Note that in the case of each nutrient series the greatest growth occurred at 65° (*cf.* fig. 11 for apple).

although there was often slight wilting at mid-day and the leaves were continually more or less curled. There must obviously have been considerable absorption of water through the old large roots. At 95° many of the large roots of both peach and apple died, but up until March 17, when the trees at this temperature were discarded, the leaves had not become seriously wilted. In harmony with these observations, there is increasing evidence in the literature (**14, 16, 2**) that plants may obtain water through a dead root system and through the large roots. CRIDER (**2**) even reports selective absorption of ions by the large non-fibrous roots of *Citrus* and *Vitis*. It may be said here, however, that nitrate was found only in the current fibrous roots of peach and apple. There was no nitrate in the old large roots either living or dead under any of the conditions of these experiments.

The obvious ability of trees to live for a considerable period in the absence of a living system of fibrous roots is a fact which should be given serious consideration. The writer has frequently found apple and peach trees in commercial orchards that were annually under adverse conditions for weeks at a time and were during that period practically devoid of living fibrous roots. Effects on the tops were often quite gradual, however, and to the casual observer not noticeable for several years.

The various causes frequently resulting in death of the fibrous roots will not be discussed here, yet it may be pointed out that effects of mineral deficiency, drought, unfavorable pH of soil, etc. are apparent in the roots of fruit trees long before the tops are seriously affected. It may also be pertinent to recall that the fine fibrous roots are the initial seat of protein synthesis in apple (**22, 30**) and probably in peach (**3, 23**).

It should be remarked, however, that brown or dead cortex does not necessarily mean a dead central cylinder. Young fibrous roots in the orchard were commonly found to be white under cool soil conditions, but the cortical tissue was invariably short lived and soon turned brown at soil temperatures above 70°. The cortex is a juvenile tissue, of course, but may remain alive and white for weeks if the soil temperature is 65° or lower. These observations in the field are therefore in apparent agreement with those already re-

corded as occurring under controlled conditions, and will be further discussed in connection with the internal structure of roots as affected by temperature.

Before considering the very different anatomical structure of the roots of the respective temperature series, certain facts concerning the relative development of callus tissue may be recorded. During the two months' period of these experiments there was practically no callus development at temperatures at 55° and below, in case of either peach or apple, although over a period of several months it probably would have occurred (**26, 29, 33**). Wound callus, however, developed rapidly at higher temperatures. It was formed earliest at 85° and 90°. At the latter temperature there was incomplete covering of the pruning wounds and the callus became completely suberized when only a few cells thick, at which time practically all cell division had ceased. There was, in general, at 85° complete covering of pruning wounds by a moderately thick layer of callus which suberized and turned brown very rapidly. At 75° the wound callus tissue was thicker and remained white for a somewhat longer time. At 65° and lower the callus tissue failed to mature. It was white and fluffy in appearance, broke off easily in handling, and the white masses of proliferating meristematic tissue continued to enlarge until the trees were harvested on April 4. There was in this respect little, if any, difference between peach and apple.

Perhaps of practical significance in propagation is the fact that the pruning wounds of the roots of peach and apple callused over more or less satisfactorily at 85°, a temperature at least 10° higher than that which could be considered favorable for root development under the conditions of these experiments. Contrary to popular opinion, roots were not observed to originate from callus tissue except in an occasional instance.

At 95° no wound callus developed, although the lenticels on living portions of the old roots became very conspicuous owing to the development of callus growth that soon suberized. This was also true at 90° and 85°, at 75° to a lesser degree, while below 75° lenticel callusing was slight. Lenticel callusing was much more conspicuous in peach than in apple. Lenticel hypertrophy seems, therefore, to be favored by an even higher temperature than that which per-

mitted abundant development of wound callus and a much higher temperature than that which permitted good growth of roots.

There appears to be nothing in the literature bearing directly on these results, although work with cuttings of apple (**26, 29**) and peach (**34**) is in harmony with the preceding observations. There is also some evidence (**26, 29**) to indicate that wound callus and especially lenticel callus is not retarded, but possibly even favored by a somewhat limited oxygen supply. For root development there seems to be required an abundance of oxygen, especially at higher temperatures. All the cultures of these experiments were provided with facilities for aeration as described under experimental methods. Nevertheless, as will be explained elsewhere, there were apparently high concentrations of carbon dioxide in the tissues of the roots at 85° and higher, which aside from direct effects must have meant less available oxygen.

## General anatomical structure of peach and apple roots

At 75° there was apparently present in both peach and apple roots the full quota of primary and secondary tissues. At other temperatures employed certain tissues did not develop or were limited in development, as will be shown later. The general structure of peach roots may therefore be most readily understood by reference to figure 5, which shows typical cross sections of young and relatively old portions of a current fibrous root as it developed at 75°. Corresponding drawings for apple roots are not shown as they were essentially the same under similar conditions.

Both genera possessed a well developed root cap, and at 1 cm. from the root tip the central cylinder was sharply differentiated from the cortex by a distinct endodermis with remarkably large casparian strips. These thickenings of the radial walls were characteristic of apple as well as peach. The cells of the root tip and those immediately adjacent were compact and angular, but the cortical cells only 1 cm. from the tip were somewhat rounded and small intercellular spaces were evident (fig. 5 *A*). Often the peripheral cells of the cortex were somewhat thicker walled just within the single layer of relatively small epidermal cells.

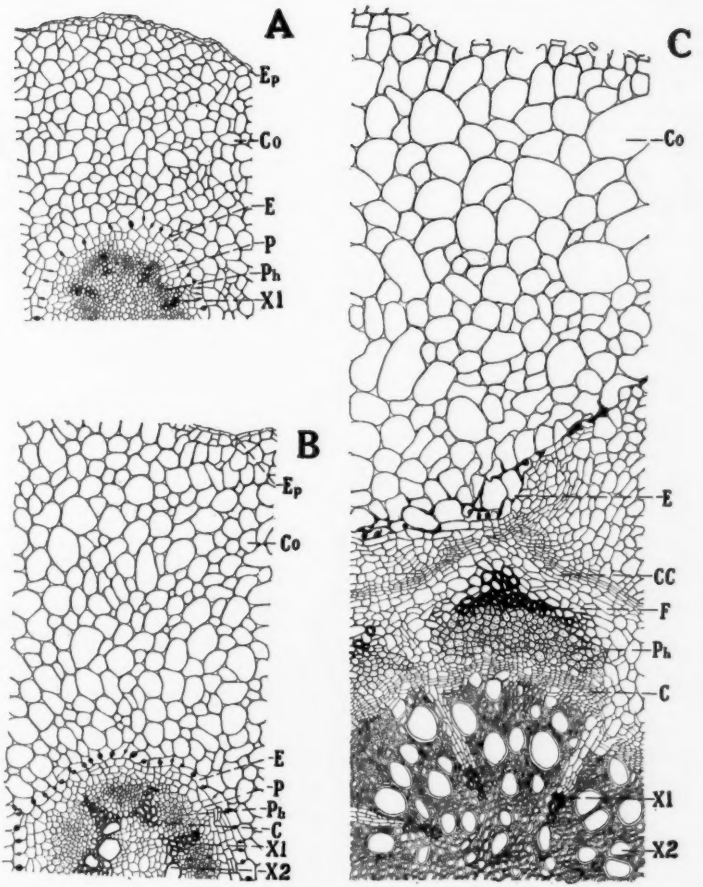


FIG. 5.*—Transverse sections of peach root grown at 75°: *A*, 1 cm. from root tip. Cortical cells not fully expanded. There are evident dividing endodermal cells, partially thickened casparian strips, primary phloem, protoxylem points, and in center of stele closely packed, thin walled cells. *B*, 5 cm. from root tip. Outer cortical cells frequently dead, although endodermis, through cell division, has accommodated itself to development of secondary phloem and xylem. Note cell division in pericycle region. *C*, 11 cm. from root tip. Entire cortex dead, strong cork cambium, heavy walled pericycle, and phloem fibers. Note portion of lenticel on right, extensive development of secondary phloem and strongly lignified xylem.

* Abbreviations for figs. 5–9: *Ep*, epidermis; *Co*, cortex; *E*, endodermis; *CC*, cork cambium; *P*, pericycle; *F*, fibers; *Ph*, phloem; *C*, cambium; *X1*, primary xylem; and *X2*, secondary xylem.

At this point and somewhat farther back there were occasionally observed a few root hairs in both genera. They were of the usual type of modified epidermal cell about the length of three or four cortical cells. Many of the roots apparently had no root hairs. They occurred so infrequently under the cultural conditions of these experiments and have been observed so seldom in the field that no definite conclusions may be drawn concerning them. Root hairs were found more often at temperatures lower than 75°, but this may have been simply because the cortex and therefore the root hairs persisted longer under cooler conditions.

At 75° the usual radial arrangement of protophloem and exarch xylem points was clearly apparent at 1 cm. from the root tip (fig. 5 *A*). In both genera the number of protoxylem groups varied from four to seven at 75°, as well as at the other temperatures employed. The extremely fine branch roots, however, usually exhibited only two or three groups each of protophloem and protoxylem.

Very fine lateral roots were most evident, however, at temperatures of 75° and higher. Note the relatively large number of fine branch roots at the higher temperatures (figs. 1–3), a situation very similar to that recorded by CONANT (1) for tobacco. The fine branch roots of apple and peach had a single layer of epidermal cells, cortical tissue only a few cells thick in cross section, an endodermis with large casparian strips, and a pericycle that showed little indication of cell division although there was some suberization in that region at 75°, just preceding death of the cortex. Shortly after emerging through the cortex of the larger "primary" root, the central cylinder of the fine laterals became lignified even to the center of the stele. There was rarely any sign of a cambium or secondary tissue. At lower temperatures there were fewer fine branch roots and most of them showed secondary thickening and consequent increase in diameter. In all other respects also they seemed similar in internal structure to "primary" roots at the same temperature.

At a point about 5 cm. back from the root tip (fig. 5 *B*) the "primary" roots at 75° usually exhibited externally a cortex that was more or less brown and often apparently dead. Invariably the cortical cells had matured as compared with those at 1 cm. from the tip, as evidenced by change in shape and increase in size of the

cells and intercellular spaces. In both peach and apple the cortex was clearly a juvenile structure, although, as will be shown later, it persisted at lower temperatures for the entire period of these experiments.

Concomitantly with approaching maturity of the cortex the endodermis increased in number of cells. The division of endodermal cells and newly developing casparian strips are shown in figure 5 *B*. Apparently the initiation of the cork cambium also took place at about the same time, as there was some indication of cell division in the region of the pericycle.

Cambial activity was likewise evident at 5 cm. from the root tip, although that the cambium had not been active long is clear from the rather small amount of secondary xylem and phloem which was present at this stage. Some of the growth of the central cylinder was due to cell enlargement. Especially in the center of the stele the cells were considerably larger than those at 1 cm. from the tip (fig. 5 *A*, *B*), but they were still rather angular, thin walled, and closely packed.

At 11 cm. from the root tip the cortex was invariably dead at 75°. Often it was completely sloughed off, although it is shown as still attached in figure 5 *C*. The torn endodermis which, at this distance from the root tip had failed to accommodate itself to the rapid growth of the central cylinder, was sometimes evident, pressed against the remains of the inner cortical tissue. The stele, however, was surrounded by an active cork cambium that produced externally an abundance of cells which suberized rapidly at 75°. Lenticels were also present at this distance from the tip. A portion of one is visible on the righthand side of figure 5 *C*. At this temperature and higher, lenticels were more numerous and larger than at lower temperatures.

Proceeding inward from the cork cambium and the more or less mature parenchymatous cells and fibers produced by it, there could occasionally be distinguished remnants of the crushed primary phloem, located of course between the fan-shaped medullary rays (fig. 5 *C*). The active cambium produced externally an abundance of secondary phloem and ray tissue far exceeding in amount that which was present at 5 cm. from the tip. Conspicuous large bundles

of strongly developed fibers were present in the region of the secondary phloem and pericycle. They undoubtedly contributed to the remarkable mechanical toughness and flexibility of these roots as already described. Also associated with the mechanical toughness and general lack of succulence, there was extensive growth of secondary xylem which was thick walled and lignified except for the xylem rays. As usual the primary rays proceeded outward from the protoxylem points; during the period of these experiments there appeared no secondary rays. In the older portion of the roots, the center of the stele, in contrast with the parenchymatous nature at a point 5 cm. from the tip, was made up of cells that had become much thickened and lignified (fig. 5 *C*).

### Effects of temperature on anatomical structure of apple and peach roots

The anatomical structure of the roots at 75° has been recorded and may be conveniently employed as a basis for comparison with the corresponding histological development at higher and lower temperatures. Because under like conditions the structural development of apple and peach was essentially the same, it will be unnecessary to discuss the two genera separately. The trees of the cultures lacking an external nitrogen supply were not seriously deficient in nitrogen, as they were initially fairly high in organic nitrogenous material (table VI). The general characteristics referred to in the following discussion apply to both plus-$NO_3$ and minus-N cultures. Certain modifications effected by added nitrate will be described elsewhere.

It has already been pointed out that at 65° and lower practically the entire current root system remained white and relatively succulent for the duration of these experiments, whereas at higher temperatures the cortex rapidly turned brown and the central cylinder tough and woody. The associated anatomical situation is indicated in figures 6–9. They were made from peach roots of the complete nutrient series which were harvested on April 4.

The rate at which tissues matured was hastened by increase in temperature. At 1 cm. from the tip at 55° there was but slight differentiation of the central cylinder, the endodermis was not yet dis-

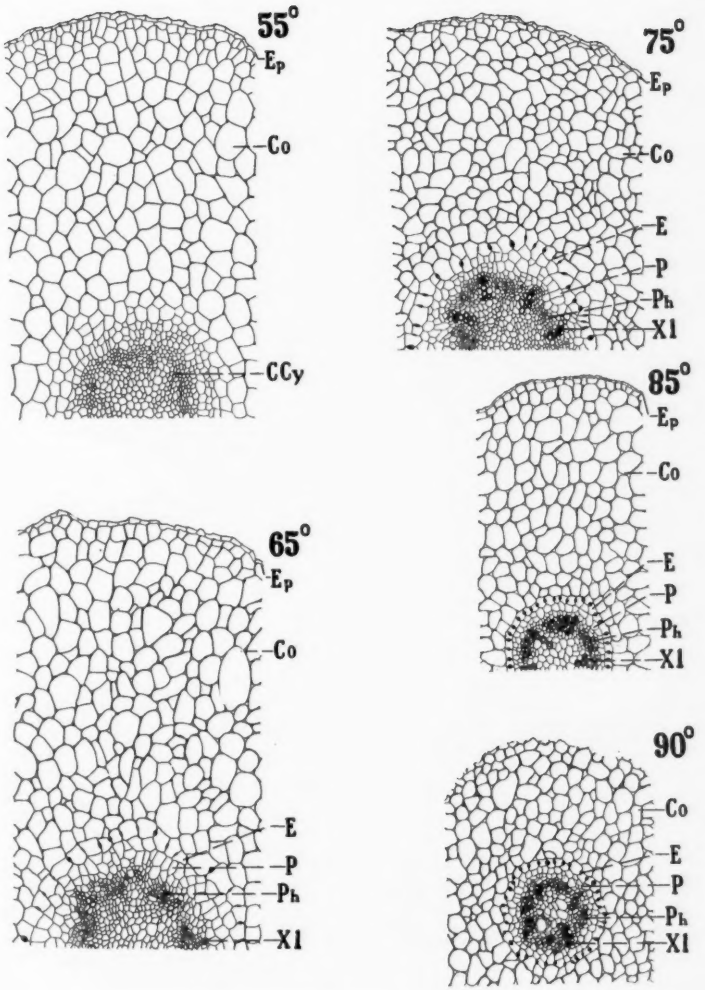


FIG. 6.—Transverse sections of peach roots 1 cm. from root tip. From low to high temperatures note decrease in number of cells and greater degree of differentiation of stele. Cortex practically dead at 85° and 90° F.

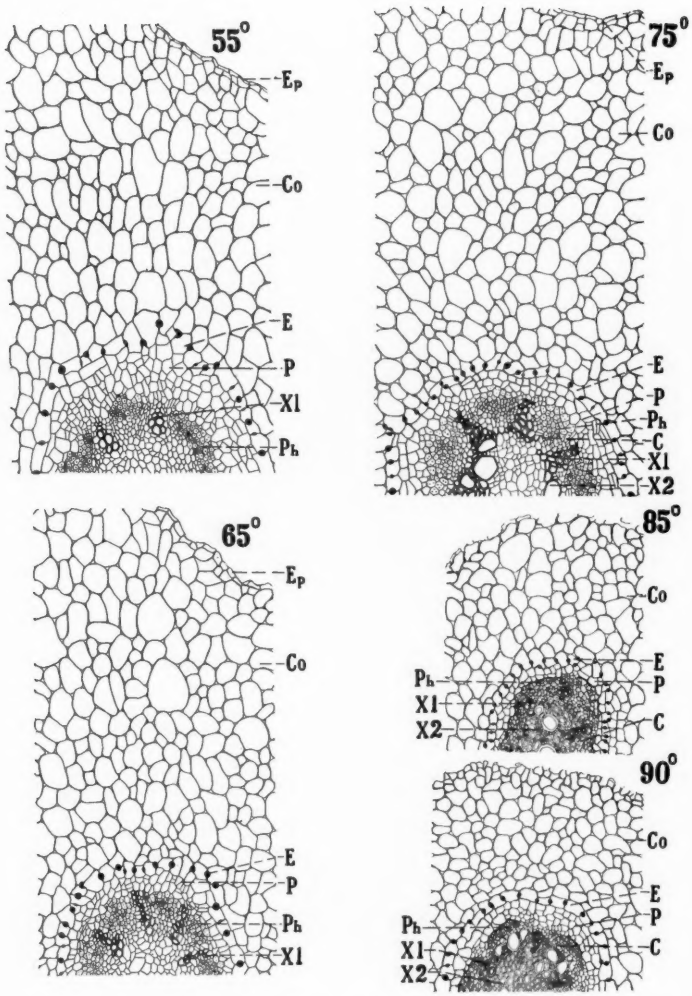


FIG. 7.—Transverse sections of peach roots 5 cm. from root tip. From low to high temperatures note greater degree of differentiation, particularly lignification of xylem, crushed primary phloem at 85° and 90°, and limited stelar cambium. Little secondary tissue below 75°. Outer cortical cells dead at 75° and entire cortex dead at 85° and 90°.

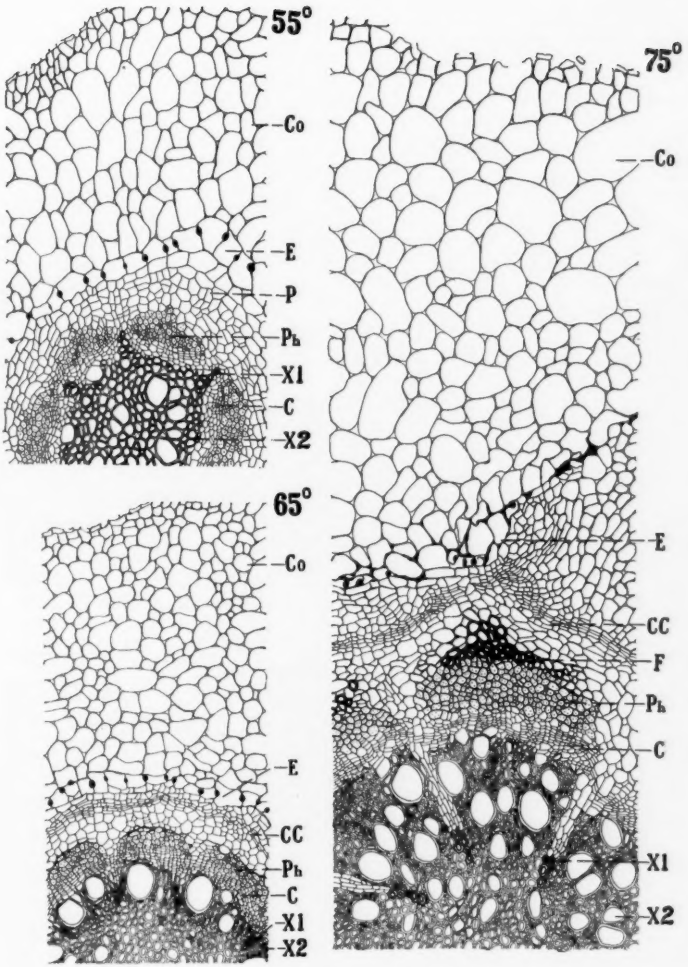


FIG. 8.—Transverse sections of peach roots 11 cm. from root tip. Cortex alive at 55° and 65° and dead at 75° (*cf.* figs. 1, 2). Note cell division in pericycle region at 55° and 65° but no suberization or fibers in contrast to well developed periderm and fibers at 75°. Primary phloem still intact at 55°, crushed at 65° and 75°. From low to high temperature note increase in amount of heavily lignified secondary xylem but presence of well developed stelar cambium at each temperature.

tinct, and the cells of the entire cross section were angular and closely packed (fig. 6). With a temperature 10° higher but at exactly the same distance from the root tip cortical cells were more mature, in that they were rounder, although still rather closely arranged. The endodermis was now distinct although the casparian strips had not reached their maximum size. In addition the central cylinder exhibited the early stages of development of the usual radial pattern of primary phloem and xylem, the latter scarcely at all lignified.

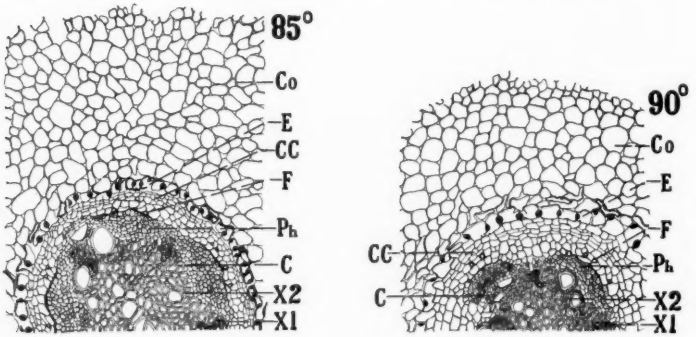


FIG. 9.—Transverse sections of peach roots 11 cm. from root tip. At both 85° and 90° cortex dead, pericyclic division limited, and periderm frequently non-continuous although cells produced externally suberized. Note at both temperatures occasional pericycle fibers, crushed primary phloem, relatively small amount of secondary phloem, and strongly lignified xylem. Amount of tissue in stelar cambium region is extremely limited, especially at 90°.

As already recorded, at 75° the protoxylem was distinctly lignified and in all other respects the roots were more advanced in maturity than at 65°. This condition was even more accentuated at 85°, and together with marked lignification of the protoxylem there were fewer cells in the potential cambium region and in the stele as a whole (fig. 6). The endodermis possessed casparian strips which at 1 cm. from the root tip were thicker than those at 75°. The cells of the cortex, although relatively small, appeared more mature, as they were much rounded, thereby resulting in large intercellular spaces.

At 90°, the highest temperature at which new current roots were produced, the total number of cells in cross section was even fewer than at 85°; and although the sections under consideration were

taken at only 1 cm. from the root tip, the cortex was not only mature but often brown and apparently dead. The endodermis showed much the same condition as at 85°, but the central cylinder, although of relatively small diameter, had advanced even further in maturity for there was lignification of both protoxylem and metaxylem, leaving in the center of the stele only a few cells which were still parenchymatous, but much rounded (fig. 6).

The preceding observations, made on cross sections taken at 1 cm. from the tip of the roots, showed clearly that with increase in temperature there was increase in rate of maturity and differentiation of primary tissues. Further, there was indication that the smaller diameter of the roots at temperatures above 75° (fig. 6) was due, in general, to fewer primary cells rather than to conspicuously smaller cells. An exception to this generality occurred at 85° and 90°, however, where the cortical cells usually died before they apparently reached their maximum potential size.

Roots of the respective series were also examined at 5 cm. from the root tip (fig. 7). At 55° there was comparatively little secondary but much primary xylem and phloem. The central cylinder as a whole consisted of relatively immature, closely packed, thin walled, angular cells. Even the small protoxylem groups were but little lignified. In the pericycle region, just inside the now conspicuous and actively dividing endodermis, there was invariably a band of tissue four or five cells wide in cross section. As shown in figure 7, these cells were angular, thin walled, and in an active state of cell division. This is in contrast to the described development at 75° of a typical cork cambium in this region. Mention should be made of the cortex, the cells of which had become rounder than at 1 cm. from the root tip, but which, like the stele, were nevertheless far from exhibiting the typical characteristics of a mature tissue. This and other observations that follow will be further discussed in connection with the chemical composition of the roots of the respective series.

At 65° the cortex showed no sign of death, but presented much the same situation as at 55°. The stele, however, was much less active in the pericycle region. It will be shown later that at relatively mature stages it exhibited a more conventional mode of pericyclic cell division than occurred at cooler temperatures. Comparatively

little, if any, secondary tissue was apparent at 5 cm. from the root tip at 65°, but the protoxylem was much more lignified than at 55°.

Unlike that of lower temperatures, the cortex at 75° was usually brown if not for the most part actually dead, except for the endodermal cells many of which were dividing. The central cylinder showed the typical initiation of a cork cambium. There was also considerable secondary phloem and xylem, the latter becoming lignified about as rapidly as produced by the cambium. Nevertheless the central portion of the cylinder still consisted of closely packed parenchymatous cells.

This is in striking contrast to the complete lignification of comparable tissue at 85° and 90° (fig. 7). At both these temperatures there was present a cambium, but in cross section it was but one or two cells wide, being bordered on the inside by thick walled xylem and on the outside by apparently mature phloem cells. In the pericycle region there was but slight indication of cell division and none at all in the endodermis. Obviously these general results support the statements already made, that with increase in temperature there was increase in rate of maturity of primary tissues.

It is proposed to describe in the following pages the development of secondary tissue of peach and apple as it occurred at the respective sand temperatures. Sections were made at exactly 11 cm. from the root tip in each case and the results are shown in figures 8 and 9.

At 55° the cortex appeared white and under the microscope looked much the same as at 5 cm. from the tip. The endodermal cells continued to divide, but perhaps unprecedented was the conspicuous band of active cells in the pericycle region as shown in figure 8. They did not show the typical organization of a cork cambium as at 75°, but seemed rather to lack regularity in their mode of division. Further they showed not the slightest sign of suberizing. They were angular and closely packed. There were no fibers in pericycle or phloem such as those occurring at 75°, and it is apparent that the remainder of the stele also was slow in maturing, for the scalloped outline of the primary xylem was still evident although the cells of the metaxylem had enlarged and become lignified. There was a wide band of active tissue in the cambium region, but it was obviously late in forming and the fact of its width indicated slow

differentiation. Consequently there was present much less secondary phloem and xylem than at higher temperatures. As already pointed out, there was produced at 55° a surprisingly large amount of primary phloem and xylem. The cells, particularly of the metaxylem, enlarged greatly before finally becoming lignified (fig. 8). These roots have already been characterized as succulent and lacking in mechanical toughness. That they were not otherwise is clear from the anatomical development just described.

At 45° and 50° the roots appeared so late that comparable material was not available for sectioning at 11 cm. from the tip. Growth proceeded far enough, however, at 50° to make it apparent that the tissues would have eventually presented much the same situation as that at 55°. The roots at 60° exhibited, as might be expected, a condition intermediate between the higher and lower temperatures.

The cortex of the roots at 65° at 11 cm. from the tip usually appeared white, although the epidermis was sometimes broken (fig. 8). In very few instances was the cortex brown or sloughed off as at 75°. Observations of the cell contents, to be described elsewhere, also confirm the view that even at 11 cm. from the tip the cortex was far from an inert tissue. The endodermis too showed indications of recent cell division for there were evident partially thickened casparian strips. In the pericycle region also there was a well defined layer of actively dividing cells, designated as cork cambium in figure 8. Nevertheless during the period of these experiments none of the cells produced by it became suberized. The stelar cambium was active and there was derived from it a considerable amount of secondary phloem and xylem; far less, however, than at 75°. Fibers of the phloem and pericycle region were essentially lacking.

The comparative immaturity of the central cylinder at all the lower temperatures and the persistence of the juvenile cortex are in contrast to the responses occurring at 75° (fig. 8). There the cortex was completely dead if not actually sloughed off, but encircling the stele was a strongly developed cork cambium which produced external cells that suberized rapidly, whereas no suberization was observed at lower temperatures for the duration of these experiments. CONANT (1) reports that in tobacco roots also, higher temperatures

favored early development of cork cambium and rapid suberization, and that there was thereby furnished an effective mechanical barrier against penetration of the fungus *Thielavia basicola*.

At 75° there occurred the greatest development of secondary tissues, including the heavy walled fibers of the phloem and pericycle region and the strongly lignified xylem. The relatively large total mass of tissues produced per "primary" root at this temperature is indicated in figure 8. It should be emphasized, however, that although this total per root was greatest at 75°, the cortex was usually dead even to within 5 cm. of the root tips; and further, that much of the stele consisted of relatively inert or at least optically empty fibers of phloem and pericycle and lignified xylem elements. It will later be seen that this fact has apparent significance in relation to metabolic responses. This relatively large mass of dead cortex, for the most part devoid of cell contents, may also help to explain why the total root system weighed more at 65° than at the higher temperature (table II). That there was also a greater number of large roots in the 65° group has been indicated (figs. 1–3).

The highest soil temperature employed by CONANT (1) was 85°, and under the conditions of his experiments it resulted in strong cork formation in tobacco roots. In apple and peach there was only very slight sign of cell division in the pericycle at 85° and 90° (fig. 9). The outer cells of the pericycle region became suberized, although not infrequently the cortex sloughed off before this occurred, especially at 90° where an occasional fibrous root died of the plus-$NO_3$ series of apple and peach. At both temperatures a few pericycle fibers developed. It is worthy of note, however, that when shifted from 60° to 95°, the already active pericycle became strongly suberized and pericycle fibers appeared in four or five days. At the same time the wide band of cells in the cambium region rapidly differentiated and, as will be explained later, changed greatly as to cell contents, after which no cell division in the cambium region was noticeable. There was differentiation of xylem and phloem practically down to the embryonic tip, which meant of course that there was little if any growth in length.

The lack of succulence of the roots at 85° and 90° is readily understood upon even casual examination of figure 9. The cortex was dead

in both lots, the condition of the pericycle has been described, and the cambium region was only one or two cells wide in cross section at 85° while at the higher temperature there was even less. In the former case, although the development of secondary phloem was not extensive, it was sufficient to crush and compress somewhat the primary phloem which appears in figure 9 as a heavy black line between the pericycle and secondary phloem. At 90° much of the primary phloem was still intact, as there was even less secondary development. Secondary xylem too was very limited in amount at 85° and 90°, but there were more and somewhat larger vessels at the lower than at the higher temperature, in both cases strongly lignified.

The general effects on anatomy of roots as caused by a shift from 60° to 95° have been mentioned. A shift from 60° to 45° caused a marked decrease in rate of growth, although the roots continued to increase considerably in length and diameter. The increase in length was made evident by a remarkably long zone of elongation between embryonic tip and region of maturation; the diametric increase was evidenced by a band of cambium unusually wide in transverse section and by pericycle several cells in width, exhibiting much the same type of cell division and lack of suberization as that described for the series continually at 55°. While at 45° the cortex persisted, there was little noticeable lignification of newly formed xylem, and in all other respects the roots were slower in maturing than those which were allowed to remain continually at 60°.

On March 17, when some of the cultures which had been continuously at 45° were shifted to 60°, current roots were entirely lacking; but by April 4 there were newly developed roots nearly equal in volume and weight and similar in external appearance and anatomy to those produced by the trees continually at 60°.

### Effects of temperature on hydrogen ion concentration of root tissues

There has been presented evidence to show that the external appearance of the roots of the respective series was intimately associated with their anatomical character, and that both were greatly modified by comparatively small differences in temperature of the

nutrient medium. Estimations of H ion concentration leave much to be desired as they give no indication of the quality or quantity of the various substances in ionic equilibrium (**10**). Nevertheless, the pH values of root tissues were found to be closely associated with the growth responses already recorded.

The conventional range indicator method (**27**) was employed and a large number of microscopic observations were made of the roots of the respective series immediately after they were removed from the sand cultures. However, it is unnecessary to present a long list of detailed observations here. The pH values given apply to both apple and peach, since respective tissues of the two genera were not noticeably different in H ion concentration under comparable conditions. The situation is briefly summed up in the following pages.

Mature xylem elements wherever observed, whether produced at continually high or low temperature, or whether developed following a shift from one temperature to another, were invariably optically empty and only the walls reacted to indicators. The pH of insoluble walls was perhaps not determined in the usual sense of the symbol, yet the reaction to such indicators as methyl red gave a so-called pH value of the lignified walls of 4.4–4.0, possibly an indication of the strength of the acids concerned. These figures would seem worth recording, however, for they were characteristic of mature xylem of the roots under the several temperature conditions. In this connection it will be recalled that lignification proceeded slowly at temperatures below 75°, whereas at this temperature and above a relatively large percentage of the root system consisted of strongly lignified xylem elements that invariably gave the acid reaction mentioned.

Cellulose walls failed to give distinct color tests, but fibers of pericycle and phloem, which it will be remembered were present only at 75° and higher temperatures, were distinctly acid. Fibers in the process of developing gave a reaction of 4.6–4.0, but some of the mature fibers were estimated to be approximately pH 3.8.

The cell contents of primary and secondary phloem were much less acid. Recently developed phloem cells regardless of their origin contained protoplasm which had a pH value of 6.2–5.9, but protoplasm of mature cells was pH 5.2–4.8. It will be recalled that the

phloem matured rapidly at higher temperatures and there was a relatively low percentage of it as compared with the rather large proportion of phloem which was present and matured slowly at temperatures below 75° (figs. 6–9), consequently the average pH of the phloem at 75° and higher was more acid than that at lower temperatures. The content of mature cells of phloem and xylem rays was consistently pH 4.4–4.0. As usual, the cellulose walls failed to give a distinct reaction.

The real pH of the stelar cambium is perhaps impossible of estimation. At 90° and 85° there was so little tissue in the cambium region, only one or two cells wide in cross section, and the protoplasmic contents were so limited in amount, that possible color reactions were masked by reflection of color from adjacent walls of the mature xylem and bordering phloem cells. At 75°, however, there was in transverse section a wide band of tissue separating xylem and phloem (fig. 5). The cells, particularly in the middle of this cambium zone, exhibited dense protoplasmic contents and were actively dividing. Freshly cut sections placed on a slide with no coverslip gave in this region a pH value of 5.2–4.8. This occurred even though the sections had been successively and rapidly washed several times with new applications of the non-alcoholic, aqueous indicator solution employed (**27**). However, in the course of half an hour or less the cambium region became much less acid, approximately 6.2–6.0, and remained so for several hours. But if a coverslip was placed over the sections the cambium region and often the phloem again exhibited a more acid reaction, pH 5.2–4.8, probably owing to accumulation in these tissues of carbon dioxide while under the coverslip. A similar observation has been made by SMALL (**27**).

The initial comparatively acid reaction may likewise have been due to carbon dioxide, but to carbon dioxide actually present in solution in the active cells of the growing roots at 75°. MAGNESS (**17**) and others (**27**) have found that when respiration is rapid, carbon dioxide may accumulate in plant tissues in amounts sufficient to account for this pH change.[3] Respiration was doubtless (**23**) much

[3] THORNTON (**31**) exposed the living hyphae of *Sclerotinia fructicola* to an atmosphere containing 20 per cent oxygen and various concentrations of carbon dioxide ranging from 10 to 60 per cent. The several treatments all resulted in a decrease in acidity of the liv-

more rapid at 75° than at lower temperatures, and there would accordingly be present relatively much carbon dioxide.

At 65° and lower the cambium did not have an initial pH as acid as that recorded for the roots at 75°, but with no coverslip had a H ion concentration of about 6.2–6.0. This would seem to indicate that there was in the growing roots a lower concentration of carbon dioxide than at 75°, owing presumably to less rapid respiration at the lower temperatures. The application of a coverslip resulted, as at 75°, in a more acid reaction of all tissues containing protoplasm, principally the cambium, young xylem, and recently developed phloem, apparently indicating that at laboratory temperature these tissues respired rapidly. A similar response was likewise obtained shortly following a shift in cultures from 60° to 95°. As will be shown later, there was available an abundance of carbohydrates for respiration at both 60° and 65°.

At 85° and 90° the pH values of cells, exclusive of the embryonic zone of the root tips, remained unaffected by the presence or absence of a coverslip, probably because of limited evolution of carbon dioxide as there were few active cells containing protoplasm. Lack of respirable material must also have been a modifying factor and will be discussed further in connection with the composition of the roots. In the embryonic zone of the root tips there was at these temperatures an apparently high concentration of carbon dioxide; at least the active tissues of this region had an initial pH of 5.0–4.8, which changed on standing with no coverslip to 5.8–5.4. Much the same situation was observed in the meristematic tissues of the root tips at 75°, but at the lower temperatures the initial and final pH was 5.8–5.6. Again the reason would seem to be that at lower temperatures respiration was slower, and therefore little carbon dioxide accumulated. That the embryonic tissues of the root tips at low temperatures were capable of rapid respiration would seem to be indicated by the increase in acidity which occurred upon leaving sections

ing hyphae from about pH 5.6 to about 7.2. The formation of carbonates if such occurred might account for this pH change, but carbonates were not determined and no explanation is as yet available. On the other hand effects on the protoplasm of an external supply of carbon dioxide might well be different from that of rapid respiration and resulting increase in carbon dioxide.

under a coverslip at laboratory temperature for a few minutes. Furthermore, shortly after shift of cultures from 60° to 95° there occurred a marked increase in acidity of all meristematic tissue, undoubtedly owing in large part to increased respiration and consequent greater content of carbon dioxide.

The real pH of meristematic tissue and of cambium may therefore be problematical, but it would seem to be less acid than that of xylem walls (4.4–4.0), and to approach the less acid condition of young phloem cells (6.2–5.9). Of course there was no clear line of demarcation between cambium and xylem. Especially at lower temperatures they intergraded insensibly one into the other. As the cells of the inner surface of the cambium differentiated, there was loss of cell contents from the developing xylem elements and the walls became more acid as lignification progressed. A more or less parallel situation also occurred in the embryonic zone of the root tips, where the corresponding mature primary tissues had about the same pH value as those which were derived from the cambium. In this connection it may again be noted that the highest percentage of meristematic tissue was found at the lower temperatures. The amount of tissue giving a less acid reaction, therefore, became increasingly less at higher temperatures where the more acid xylem elements and fibers of phloem and pericycle predominated.

At 75° and higher, the pericycle on its inner surface produced fibers already described and parenchymatous cells which had a pH value of 5.2–4.8. On the outer surface of the pericycle the walls of the suberized cells showed a reaction to indicators of 4.2–4.0. The actual pH of the cork cambium could not be determined on account of reflection of colors from the adjacent tissues produced by it. The pH value was probably not much, if any, less acid than 5.2, as otherwise indicators negative in reaction to adjacent tissues would have given a positive test in the cork cambium region.

The endodermis was among the first structures of the root to be clearly differentiated, and from that time until maturity the cell contents exhibited a pH value of 4.4–4.0. The casparian strips also gave a positive reaction at approximately the same H ion concentration. There was no noticeable difference in this respect between

roots of the several series, and the same values were maintained as long as the endodermis persisted.

Most of the cells in the remainder of the cortex were much less acid (5.2–4.8), although there was a considerable number of scattered cortical cells, singly or in small groups, which gave a reaction of 4.4–4.2. The contents of such cells are described later. As the cortex matured, the protoplasmic content decreased and the proportion of relatively acid cells became higher. When dead it was practically devoid of cell contents and the brownish walls gave no noticeable reaction. It will be recalled that at 65° and lower all cortical tissue persisted for the duration of these experiments, whereas at higher temperatures the cortex matured rapidly and died.

At temperatures of 75° and higher, relatively young cortex from sections made near the root tips exhibited an initial reaction of about 4.4, but on standing for a few minutes the majority of the cells became less acid. This was undoubtedly due in part to the so-called "wound carbon dioxide," but was nevertheless much more pronounced at 75° than at 65° or lower. It probably was associated with higher carbon dioxide content and more rapid respiration in the roots grown at higher temperatures.

The epidermis of washed roots apparently had a more or less permanent pH value of about 4.4–4.2, but the surface of the current roots as they existed in the sand cultures was modified by the nutrient treatment. The trees lacking nitrogen in the culture solution had a pH value at the unwashed root surfaces of about 4.4–4.2, approximately that of the minus-N nutrient solution as applied. For trees receiving nitrate the corresponding value varied from 4.8 to 6.0. The less acid condition was found where nitrate was being absorbed and assimilated most rapidly, and was undoubtedly due, at least in part, to more rapid absorption of nitrate than of basic ions by the roots under the conditions of these experiments. This point has been discussed in connection with the results of other work (**19**).

The observations so far recorded show that the rapidly maturing non-succulent roots at temperatures of 75° and higher lost their cortex early, and were comparatively high in proportion of strongly lignified xylem, suberized tissue of the periderm, and mechanical fibers of phloem and pericycle, all of which exhibited an acid reac-

tion. On the other hand they were low in proportion of less acid tissues, such as young phloem and cambium, and the embryonic zone of the root tips was also very limited in volume. Such meristematic tissues as were present were relatively acid, owing apparently to a high content of carbon dioxide coupled presumably with a high rate of respiration.

In contrast, the roots at lower temperatures were characteristically succulent and the cortex persisted. The stele was slow in differentiating and consisted of relatively much active tissue that was notably less acid than mature tissues, a pericycle that was extremely active but did not suberize or produce fibers, phloem that was likewise entirely lacking in mechanical elements, and cambium which was in an active state of cell division but produced on its inner surface xylem that lignified and reached its maximum degree of acidity very slowly.

All the foregoing facts on anatomy and pH of tissues have an important bearing upon the observations which are recorded in the following pages.

## Effects of temperature on absorption and assimilation of nitrate by apple and peach roots

Under the conditions of these experiments, temperature did not materially limit the ability of the trees to absorb nitrate. Some of the apple and peach trees lacking nitrate were later supplied with the complete nutrient solution (table I). On the basis of nitrate appearance within the current roots of these trees no consistent difference in permeability could be detected. It was only a matter of minutes after application before the entire current root system of both apple and peach contained nitrate in abundance, regardless of the temperature of the sand in which the trees were growing.

Without exception, nitrate was limited exclusively to the current roots for the entire period of these experiments. Why nitrate was found only in these organs is not clear, but inorganic nitrogen during most of the year is commonly limited to the fibrous roots of apple (**7, 19, 22, 30**), peach (**3, 23**), and other perennial plants (**20, 21**). Davidson and Shive (**3**) found that nitrate was present, however, in the aerial organs of peach when an alkaline reaction of the nu-

trient medium apparently prevented (**3, 19, 32, 33**) utilization of nitrate by the roots. STUART (**28**) likewise reports nitrate in the tops of young apple trees which received excessive applications of nitrate and exhibited leaf injury. ECKERSON (**7**) and THOMAS (**30**) found nitrate in the tops of apple trees but only in the buds as they were opening. ECKERSON states, "It seems safe to conclude that if there is nitrate in the soil some will be carried to the buds when they begin to swell in the spring, and for a short period the buds (and adjacent bark) take an active part in the reduction of nitrate and synthesis of amino acids." ECKERSON'S work and that of others cited shows clearly that the fibrous roots are the organs chiefly concerned in the assimilation of nitrate nitrogen. Under the conditions of these experiments they were apparently the only organs of apple and peach that were engaged in this phase of nitrogen metabolism.

Whereas the temperature of the nutrient medium affected little, if at all, the ability of the roots to absorb nitrate (provided they were not already saturated to capacity with it), temperature had a very marked influence on the capacity of the roots to reduce nitrate to nitrite, ammonium, and amino acids. This process of reduction and synthesis may be conveniently included in the term *nitrate assimilation*. The relative rate at which this phase of protein synthesis takes place is apparently indicated with a fair degree of accuracy by ECKERSON'S method (**6**), which briefly consists of taking an aqueous extract of fresh plant tissue and measuring the amount of nitrite reduced from nitrate by a given sample under specific conditions of time, pH, and temperature. The amount of nitrite formed from nitrate gives a measure of the *reducase* activity of the particular plant or organ sampled. Analyses have repeatedly shown (**6, 8, 18, 22**) that reducase activity closely parallels the synthesis in the plant of amino acids and other forms of elaborated nitrogen. Reducase activity therefore furnishes an index of the relative rate of nitrate assimilation.

### *Nitrate assimilation by apple*

Aliquots of the entire current root system of apple were employed during the early part of these experiments and determinations made of reducase activity. When the first harvest took place during early

March, reducase activity seemed to be roughly proportional to temperature, the maximum reduction occurring at higher temperatures (fig. 10). But as the experiments progressed, at temperatures of 75° and higher, there occurred a marked decrease in ability of the roots to reduce nitrate; whereas at 65° and lower the respective curves for reducase activity dropped very little.

Such a response does not seem surprising, however, since at higher temperatures, with each later harvest, the samples of whole current roots became increasingly high in proportion of dead cortex and relatively inert xylem along with mechanical elements of phloem and pericycle. It will be recalled that at 65° and lower the cortex persisted, differentiation of lignified xylem was slow, and no fibers appeared in either phloem or pericycle. In addition, the respective tissues at lower temperature were relatively less acid, a fact probably of considerable significance. A slightly alkaline condition greatly accelerates nitrate reduction in the plant, at least in the case of tomato (**5**).

It therefore appeared probable that at higher temperatures reduction of nitrate occurred principally in recently developed and still active portions of the root system which were comparatively less acid. For a comparison of nitrate reduction at different temperatures, samples were accordingly taken in the vicinity of the root tips, care being observed to avoid any possibility of desiccation. The length of the so-called root tips varied from 3 to 5 cm. The chief object was not to secure samples of any particular length, but to obtain recently developed tissue that still contained a high proportion of cells with comparatively dense protoplasmic contents.

The aliquots of entire current roots of the early harvests were approximately in this category as they had been growing for but a short period. The reducase determinations on these, together with those for the root tips harvested on subsequent dates, gave the results for reducase shown in figure 10. The curves vary somewhat. They are not straight lines but they show beyond any reasonable doubt that the rate of nitrate reduction increased with increase in temperature. They show too that it varied little from week to week under these conditions where there were present cells which contained abundant protoplasm. Samples for reducase determinations were likewise

taken from the proximal or oldest end of roots of the respective series. The results are not reported in detail as they simply corroborate those already recorded. At 75° and higher, where the cortex was completely dead and the central cylinder in large part made up of apparently inert tissues, such as lignified xylem and fibers of phloem and pericycle, there was practically no nitrate reduction, a finding seemingly in accord with the work of ECKERSON (**7**) who reports little or no reducase in the larger fibrous roots of apple. In contrast, at 65° and lower, where the cortex was still alive and the stele much less mature (figs. 6–9), reducase activity at any given temperature was nearly as high at the proximal as at the distal end of the root.

Reducase determinations were also made before and after shifting the apple trees from one temperature to another. The results are expressed in milligrams of nitrogen as nitrite per gram of fresh tissue. On March 17, the roots at 60° had a value of 0.14 mg. of nitrite nitrogen, but at the beginning of the third day after shifting to 95° the corresponding figure was 0.20, two days later 0.07, and by the last of March there was practically no nitrate reduction. Obviously a temperature even as high as 95° increased reducase activity but only for a comparatively brief period, while protoplasm was still relatively abundant. It will be recalled that shortly following the shift from 65° to 95° there was rapid differentiation of tissues, lignification of xylem, and death of the cortex. Essentially the same response occurred in another set of experiments with apple (**22**) and likewise with tomato (**18**) when shifted from a relatively low temperature to 95°.

On the other hand, some of the apple trees of the group at 60° were shifted on March 17 to a sand temperature of 45°. Just before shifting and at intervals until April 4, reducase tests were made. In chronological order the results were: on March 17, 0.14; on following dates 0.12, 0.10, 0.05, and 0.13 mg. of nitrite nitrogen per gram of fresh weight of the current roots. There was thus apparently maintained a comparatively high plane of reducase activity in these roots, which matured little if any following the shift to low temperature, and which before being transferred from 60° to 45° were fairly high in nitrate reducing ability.

Previous work (**22**) with the same variety of apple (Stayman)

showed that there was apparently synthesis of amino acids by the current roots during a period in which the trees were held at 45°. It may be remarked, however, that the organic products of nitrate reduction largely remained in the roots in case of Stayman, whereas Baldwin translocated the newly synthesized products of nitrate reduction to the tops. Both roots and aerial organs were subjected to the same temperature.

It has been pointed out that the rate of nitrate reduction increased with rise in temperature, even to 95°, the highest employed in these experiments. This was true, however, only when relatively young active tissues were employed for testing. Low temperature favored the production of roots which were proportionately high in such tissues, but at high temperatures the cortex died early and in the stele the predominating tissues were woody, lacking in protoplasm and in ability to reduce nitrate (figs. 6–9).

It seemed logical, therefore, to find that the current root system at the intermediate temperature of 65° was highest in absolute reduction of nitrate as computed on the arbitrary basis of nitrite produced *in vitro* (fig. 11). It is true that the greatest volume and weight of roots were produced at this temperature, and that none of the tissues included in the yield recorded were dead, as was much of the cortex at higher temperatures. But it is not without significance that the roots were comparatively high in proportion of active tissues and low in relatively inert elements, such as mechanical fibers, lignified xylem, and strongly suberized periderm.

In this connection it may be noted that the curves for fresh weight of current tops produced at the respective sand temperatures (fig. 11) closely paralleled the curves for absolute reducase activity per current root system. A little consideration makes it appear doubtful, however, whether a continuous temperature of 65° would be desirable, for the root systems grown at that temperature were lacking in mechanical strength as compared with those at 75°.

### *Nitrate assimilation by peach*

Before considering the chemical composition of the current roots, it should be stated that negative results were obtained in all attempts to demonstrate reducase activity in peach. The extracts of

current roots, old roots, old stem, and current tops were adjusted to various pH values in addition to the usual one of 7.2. Cyanogenetic material was also removed by hydrolysis with emulsin with accompanying aspiration into receiving flasks containing alkali (**4**), following which the cyanide-free extracts were tested in the usual manner for reducase activity, but no nitrate reduction occurred.

These tests were conducted over a period of only two weeks, using as material Elberta peach trees that had been grown under the greenhouse conditions of December and January in sand culture at a temperature of 60°–70°. The current roots were in external appearance, anatomy, and composition much like those of the groups grown continually at 65°. Occasional reducase tests were made on peach trees during the course of the experiments, but the extracts failed to give evidence of nitrate reduction. The lack of positive results may mean little, however, as the trials did not cover a sufficient period of time in the growth cycle of peach. Apple may exhibit little or no reducase activity for a considerable length of time as grown under commercial conditions in the orchard (**7**). In case of the plant material available, it therefore seemed more expedient in these particular experiments to study nitrate assimilation in peach by microchemical observation, and by measurements of the assimilated or nitrate-free nitrogen present in the current roots before and after addition of nitrate. The analytical data so secured are given in tables V and VI. These data and the current growth of tops obtained (fig. 4) made it appear that the general course of nitrate reduction in peach was essentially the same as in apple under comparable conditions.

Also, strong nitrite reactions were obtained only in the current roots of both genera. This product of nitrate reduction appeared in about 24 to 36 hours at 60° and 65°, and in from 12 to 18 hours at 75°. At higher and lower temperatures nitrite tests gave negative results, possibly because present in extremely small amount at any one time, or nitrite may have been present for a brief period during which time the plant material was not examined. Even at the intermediate temperature of 65°, nitrite was, as usual (**5, 18**), present for a period of only a few hours, shortly following the initial applica-

tion of nitrate. Nitrite is not generally found in quantity in plants continually supplied with an external source of readily available nitrate.

## Effects of temperature on chemical composition of apple and peach roots

### *Nitrogenous material in root systems of apple*

The apple trees were continually supplied with the complete or plus-$NO_3$ nutrient solution. The results of analyses of the current roots are given in table III. At the extremes of temperature employed there was insufficient tissue for macroanalysis.

TABLE III

Stayman apple trees
Dry matter, carbohydrates, nitrate, and nitrate-free nitrogen in current fibrous roots on April 4, expressed as percentage of ash-free green matter

| Sand temperature (°F.) | Dry matter | Reducing sugars | Sucrose | Starch and dextrin | Nitrate-free nitrogen | Nitrate nitrogen |
|---|---|---|---|---|---|---|
| 50* | 6.8 | 0.13 | 0.08 | 0.50 | 0.41 | 0.012 |
| 55 | 7.3 | 0.09 | 0.11 | 0.39 | 0.45 | 0.014 |
| 60 | 7.0 | 0.10 | 0.06 | 0.71 | 0.30 | 0.010 |
| 65 | 9.0 | 0.11 | 0.07 | 1.04 | 0.27 | 0.009 |
| 75 | 12.0 | 0.03 | 0.00 | 0.83 | 0.13 | 0.011 |
| 85 | 15.2 | 0.01 | 0.00 | 0.20 | 0.09 | 0.016 |
| 60–45† | 7.5 | 0.17 | 0.12 | 1.74 | 0.26 | 0.017 |
| 60–95† | 11.7 | 0.03 | 0.00 | 0.42 | 0.14 | 0.013 |

* Amount of current root growth was inadequate for macro-analysis at 45°, 90°, and 95°.
† Shifted March 17.

It is obvious that there was an abundance of nitrate in the current roots of each group of trees, and that the difference in concentration of nitrate under the several temperatures was perhaps too small to be significant. It may be noted, however, that at 65°, where reducase activity was highest (figs. 10, 11), the nitrate content was lowest and it was somewhat higher at 85° and 50° where the assimilation of nitrate was less vigorous. Likewise, when some of the trees at 60° were shifted to 45° and 95° respectively, the percentage of nitrate nitrogen increased materially. It is not uncommon for nitrate to ac-

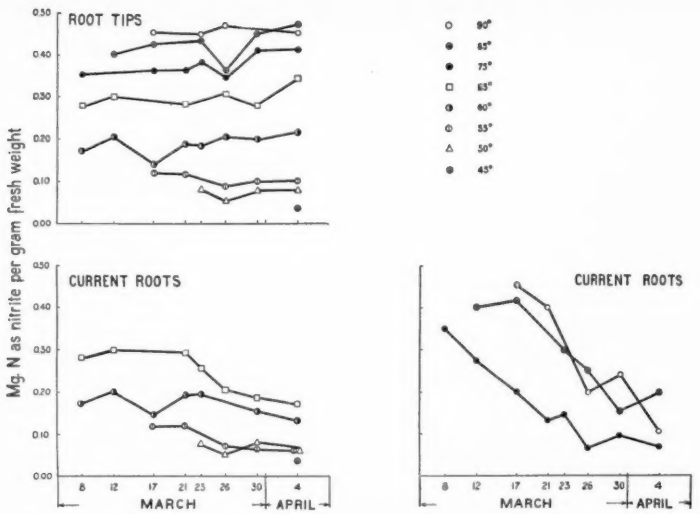


FIG. 10.—Reduction of nitrate by extracts from tips of apple roots and by extracts from aliquots of complete current root system. Roots grown at temperatures indicated, the tops at about 65° F.

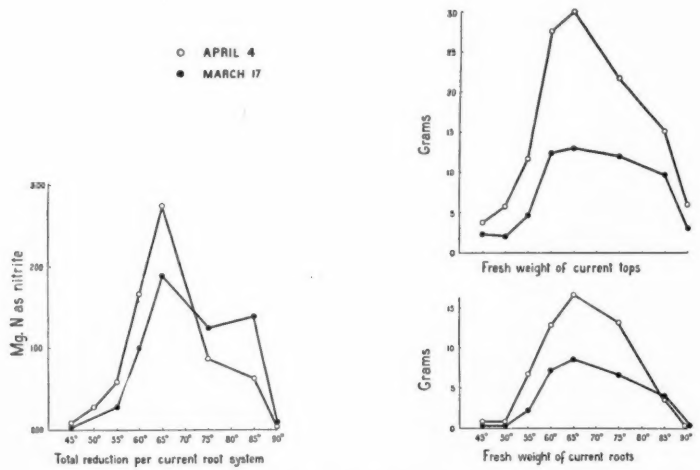


FIG. 11.—By computation, absolute reduction of nitrate by entire current root system of apple and fresh weight yield of current roots and tops. The aerial organs were grown at about 65°, the roots at temperatures indicated.

cumulate in plants when there is little utilization of it in protein synthesis.

The column of percentage figures under the heading Nitrate-free nitrogen (table III) furnishes an index to the concentration of organic nitrogen, including such compounds of elaborated nitrogen as asparagine, amino acids, and proteins. It is evident that the low temperatures of these experiments were associated with the greater concentrations and high temperatures with the lower concentrations of organic nitrogen in the current roots. Even at 65°, although reducase activity was greatest here (fig. 10), there was much less assimilated nitrogen, on a percentage basis, than at lower temperatures.

In view of the results on reducase activity, these data might seem unreasonable if there was not available information concerning the anatomical structure of the root systems concerned. Meristematic and relatively undifferentiated tissues are notably high in proteins and they were the predominating type of tissue at lower temperatures. It will be recalled that differentiation was slow, that the cortex persisted, and that much of the stele remained parenchymatous for a considerable time and lost protoplasm (incidentally proteinaceous material) very slowly at temperatures lower than 65°. At 75° and above the reverse situation occurred; there the relatively high proportion of dead cortex, necessarily included with the living tissue for chemical analysis, was essentially devoid of all cell contents and the stele consisted in large part of relatively inert elements such as suberized periderm, mechanical fibers of pericycle and phloem, and strongly lignified, optically empty xylem. All these tissues were presumably low, if not entirely lacking, in organic nitrogen and gave no positive tests for it.

In these experiments the high percentage of organic nitrogen was intimately associated with the comparatively immature condition of the roots. This should not be confused with the results of earlier work (**22**) in which accumulation of organic nitrogen occurred in root systems of Stayman because at 45° the newly synthesized amino acids were apparently not freely translocated to the tops. During the brief period that the trees were held at that temperature the initially present root systems there concerned were relatively

more mature in anatomical character and therefore lower in proportion of meristematic tissues. Nitrogen was there stored in large part as amino acids and asparagine. In the present experiments much of the organic nitrogen of the roots at low temperature was in the form of complex proteins of the cambium, pericycle, phloem, and of the extensive embryonic zone of the root tips.

The shift from a sand temperature of 60° to 45° in these experiments had no appreciable effect upon the percentage of organic nitrogen in the current roots (table III). The tops were grown at an air temperature of about 65°, however, and there may well have been translocation of organic nitrogen to the actively growing leaves and stem. In the previous experiments just cited (**22**) there was practically no growth of tops when the air as well as the sand was held at 45°.

Some of the trees of the 60° group were likewise shifted to a sand temperature of 95°. It will be recalled that shortly following the transfer there occurred differentiation of the stele and death of all cortical tissue nearly to the root tip. This marked decrease in amount of embryonic and relatively immature tissue with increase in proportion of heavily lignified xylem and mechanical elements of the periphery of the stele resulted in the marked drop in concentration of organic nitrogen shown in table V.

Concomitantly with differentiation of tissues and decrease in amount of protoplasm, complex proteins decreased, but there was no noticeable accumulation of proteolytic material. The products of protein hydrolysis were probably rather rapidly translocated to the tops; at least the terminal leaves temporarily became relatively soft and dark green and the developing internodes comparatively long and spindling. Microscopic observations also showed that the tips of the stems and especially the petioles were high in concentration of soluble organic nitrogen.

As already mentioned, no nitrate was found in the old roots, but it is significant that the percentage of organic nitrogen in these initially present structures (table IV) corresponded closely in trend to the curve for absolute reduction of nitrate as shown in figure 11. The detailed analyses of the tops are not reported, but a similar

situation was found in the old stems, current stems, and leaves. The growth of tops (fig. 4) and the organic nitrogen content of aerial organs and old roots (table IV) thus practically paralleled the curve for absolute reducase activity of the respective current root systems (fig. 11), tending to show that the reducase determinations furnished a fairly accurate index of effects of temperature on nitrate assimilation as described in preceding pages.

TABLE IV

STAYMAN APPLE TREES
DRY MATTER, CARBOHYDRATES, AND NITRATE-FREE NITROGEN IN OLD ROOTS ON APRIL 4, EXPRESSED AS PERCENTAGE OF GREEN MATTER

| SAND TEMPERATURE (°F.) | DRY MATTER | REDUCING SUGARS | SUCROSE | STARCH AND DEXTRIN | NITRATE-FREE NITROGEN* |
|---|---|---|---|---|---|
| 45 | 58.00 | 1.59 | 0.71 | 5.55 | 0.051 |
| 50 | 50.00 | 1.91 | 0.80 | 4.00 | 0.054 |
| 55 | 45.20 | 0.94 | 0.33 | 4.17 | 0.072 |
| 60 | 43.33 | 0.70 | 0.44 | 4.76 | 0.081 |
| 65 | 43.33 | 0.83 | 0.38 | 4.30 | 0.070 |
| 75 | 38.86 | 0.54 | 0.25 | 3.12 | 0.068 |
| 85 | 34.61 | 0.71 | 0.38 | 2.00 | 0.050 |
| 90 | 42.70 | 0.83 | 0.32 | 4.11 | 0.055 |
| 95 | 43.00 | 0.50 | 0.27 | 4.29 | 0.060 |
| (Initial Feb. 1) | (57.80) | (1.82) | (0.77) | (6.07) | (0.052) |

* No nitrate was found in the old roots of any of the trees at any time.

### *Nitrogenous material in root systems of peach*

All of the peach trees were grown with no external supply of nitrate until March 17. At that time the foliage was fairly dark green and there was little, if any, evidence of nitrogen deficiency as the old roots and old stem initially contained an abundance of nitrogen (table VI). As already stated, the volume of current top growth followed closely in trend the production of roots at the respective sand temperatures. Nitrate applied on the date mentioned was immediately absorbed in liberal amount in all cases and was limited to the current roots. The percentage of nitrate nitrogen in these organs at the time of final harvest on April 4 is shown in table V. The variations in concentration of nitrate at the several temperatures were

small, but at 85°, 90°, and in the set shifted from 60° to 95°, nitrate is relatively high, possibly because of limited synthesis of organic nitrogen. At least the addition of nitrate resulted in practically no increase in organic nitrogen in the new or in the old roots (tables V and VI) of the cultures concerned.

The concentration of nitrate-free nitrogen in the respective current root systems on March 17 is not low, even though there was available no external supply of nitrogen. The initially present old roots and old stems must obviously have furnished the entire amount of nitrogen which contributed to the growth of new roots and aerial organs. It was undoubtedly made available mainly through proteolytic changes and subsequent translocation to current roots and tops.

Some of the peach trees which received the nutrient solution lacking nitrogen were harvested for analysis on March 17 (table V). As in apple, the concentration of nitrate-free nitrogen in the current roots was found to be highest at low temperatures. This was not because there was more rapid proteolysis or greater reutilization of stored proteins under cooler conditions. The amount of organic nitrogen in the current root systems at low temperatures was obviously not great as the amount of root growth was relatively little (table II; figs. 1, 2). The high percentage of organic nitrogen was associated with the high proportion of relatively meristematic tissues present at 65° and lower. Similar responses occurred in apple and have already been discussed in considerable detail.

Essentially the same situation was found when the remainder of the trees which lacked nitrate were harvested for analysis on April 4 (table V). The only result possibly requiring comment is the relatively high percentage of organic nitrogen in the current roots following the shift from 60° to 95°. This probably does not represent an increase in absolute amount of organic nitrogen so much as an increase in percentage due to loss in carbohydrates (table V). Nevertheless, with the marked drop in dry matter and carbohydrates which occurred in the old roots following the shift (table VI), there may well have been hydrolysis of stored proteins in the old roots and subsequent translocation of the proteolytic products to the current roots. At least with decrease in dry matter the old roots, instead of

## TABLE V

ELBERTA PEACH TREES

DRY MATTER, CARBOHYDRATES, NITRATE, AND NITRATE-FREE NITROGEN IN CURRENT FIBROUS ROOTS EXPRESSED AS PERCENTAGE OF ASH-FREE GREEN MATTER

| Sand temperature (°F.) | March 17† | | | | | April 4 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Minus-N | | | | | Minus-N | | | | | Plus-$NO_3$† | | | | |
| | Dry matter | Reducing sugars | Sucrose | Starch and dextrin | Nitrate-free nitrogen | Dry matter | Reducing sugars | Sucrose | Starch and dextrin | Nitrate-free nitrogen | Dry matter | Reducing sugars | Sucrose | Starch and dextrin | Nitrate-free nitrogen | Nitrate nitrogen |
| 45 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 5.1 | 0.39 | 0.20 | 0.03 | 0.19 | 0.09 |
| 50 | 5.2 | ..... | ..... | ..... | ..... | 4.9 | 0.76 | 0.29 | 0.11 | 0.19 | 6.8 | 0.59 | 0.17 | 0.20 | 0.20 | 0.08 |
| 55 | 5.0 | 0.84 | 0.17 | 0.24 | 0.19 | 5.2 | 0.61 | 0.20 | 0.40 | 0.17 | 9.3 | 0.33 | 0.09 | 0.30 | 0.22 | 0.09 |
| 60 | 7.1 | 0.93 | 0.27 | 0.30 | 0.18 | 8.0 | 0.50 | 0.17 | 0.67 | 0.18 | 8.8 | 0.07 | 0.02 | 0.33 | 0.24 | 0.08 |
| 65 | 7.3 | 0.80 | 0.11 | 0.51 | 0.15 | 9.4 | 0.55 | 0.21 | 0.75 | 0.10 | 7.2 | 0.11 | 0.05 | 0.21 | 0.27 | 0.07 |
| 75 | 8.5 | 0.27 | 0.09 | 1.00 | 0.10 | 7.0 | 0.19 | 0.08 | 0.87 | 0.07 | 12.5 | 0.11 | 0.00 | 0.90 | 0.26 | 0.10 |
| 85 | 11.8 | 0.16 | 0.09 | 0.85 | 0.09 | 13.0 | 0.03 | 0.00 | 0.74 | 0.05 | 15.5 | 0.08 | 0.04 | 1.00 | 0.08 | 0.12 |
| 90 | ..... | ..... | ..... | ..... | ..... | 14.1 | 0.04 | 0.01 | 0.29 | 0.05 | ..... | ..... | ..... | ..... | ..... | ..... |
| 60–45* | (7.1) | (0.93) | (0.27) | (0.30) | (0.18) | 10.1 | 0.82 | 0.41 | 1.39 | 0.15 | 11.3 | 0.65 | 0.09 | 1.44 | 0.14 | 0.07 |
| 60–95* | (7.1) | (0.93) | (0.27) | (0.30) | (0.18) | 12.5 | 0.27 | 0.00 | 0.09 | 0.23 | 11.4 | 0.09 | 0.04 | 0.12 | 0.26 | 0.16 |
| 90–60* | ..... | ..... | ..... | ..... | ..... | 13.0 | 0.23 | 0.04 | 1.00 | 0.11 | 10.9 | 0.18 | 0.09 | 0.39 | 0.17 | 0.05 |

* Some of the trees were shifted March 17.

† Prior to March 17 the trees received no external nitrogen supply and contained no nitrate. On that date some of them received nitrate.

TABLE VI

ELBERTA PEACH TREES

DRY MATTER, CARBOHYDRATES, AND NITRATE-FREE NITROGEN IN OLD ROOTS EXPRESSED AS PERCENTAGE OF GREEN MATTER

| SAND TEMPERATURE (°F.) | MARCH 17 | | | | | APRIL 4 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MINUS-N | | | | | MINUS-N | | | | | PLUS-$NO_3$† | | | | |
| | DRY MATTER | REDUCING SUGARS | SUCROSE | STARCH AND DEXTRIN | NITRATE-FREE NITROGEN‡ | DRY MATTER | REDUCING SUGARS | SUCROSE | STARCH AND DEXTRIN | NITRATE-FREE NITROGEN‡ | DRY MATTER | REDUCING SUGARS | SUCROSE | STARCH AND DEXTRIN | NITRATE-FREE NITROGEN‡ |
| 45 | 49.5 | 1.2 | 0.9 | 5.0 | 0.041 | 49.0 | 1.5 | 0.5 | 5.3 | 0.040 | 49.3 | 1.7 | 0.8 | 6.0 | 0.038 |
| 50 | 50.0 | 1.3 | 0.8 | 4.8 | 0.050 | 49.0 | 1.7 | 0.8 | 5.2 | 0.040 | 45.0 | 1.4 | 0.9 | 5.4 | 0.041 |
| 55 | 48.2 | 1.1 | 1.0 | 5.2 | 0.042 | 43.0 | 1.5 | 0.8 | 5.7 | 0.042 | 40.0 | 1.7 | 0.7 | 5.0 | 0.050 |
| 60 | 47.0 | 0.9 | 0.6 | 4.5 | 0.053 | 42.5 | 0.7 | 0.4 | 4.0 | 0.048 | 40.8 | 1.0 | 0.4 | 2.8 | 0.061 |
| 65 | 46.0 | 1.0 | 0.2 | 4.1 | 0.061 | 41.7 | 0.8 | 0.7 | 5.5 | 0.050 | 39.5 | 0.6 | 0.5 | 3.0 | 0.066 |
| 75 | 44.1 | 0.8 | 0.4 | 4.0 | 0.058 | 39.3 | 0.2 | 0.0 | 4.2 | 0.062 | 38.5 | 0.3 | 0.1 | 2.1 | 0.001 |
| 85 | 46.0 | 0.7 | 0.3 | 2.9 | 0.062 | 40.0 | 0.1 | 0.0 | 2.1 | 0.060 | 42.0 | 0.2 | 0.2 | 1.9 | 0.068 |
| 90 | 47.0 | 0.5 | 0.8 | 5.4 | 0.044 | 42.6 | 0.5 | 0.4 | 6.0 | 0.040 | 43.0 | 0.9 | 0.3 | 5.0 | 0.040 |
| 95 | 47.3 | 0.9 | 0.8 | 5.0 | 0.045 | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... |
| 60–45* | (47.0) | (0.9) | (0.6) | (4.5) | (0.053) | 46.6 | 0.6 | 1.2 | 5.2 | 0.050 | 40.0 | 0.8 | 0.6 | 5.0 | 0.048 |
| 60–95* | (47.0) | (0.9) | (0.6) | (4.5) | (0.053) | 45.0 | 0.7 | 0.4 | 4.8 | 0.050 | 48.0 | 0.9 | 0.5 | 4.9 | 0.052 |
| 90–60* | (47.0) | (0.5) | (0.8) | (5.4) | (0.044) | 45.2 | 1.2 | 1.0 | 5.0 | 0.051 | 44.0 | 0.8 | 0.6 | 5.2 | 0.048 |
| 45–60* | (49.5) | (1.2) | (0.9) | (5.0) | (0.041) | 46.8 | 0.7 | 0.4 | 4.1 | 0.050 | 42.0 | 0.7 | 0.5 | 3.0 | 0.062 |
| (Initial trees Feb. 1) | (52.8) | (1.4) | (1.0) | (4.8) | (0.040) | | | | | | | | | | |

* Trees shifted March 17.

† Prior to March 17 the trees received no external nitrogen supply and contained no nitrate.

‡ No nitrate was found in the old roots of any of the trees at any time.

gaining, lost slightly in concentration of organic nitrogen (table VI). As in the case of the apple trees subjected to the same shift, the peach trees displayed a sudden and temporary increase in rate of growth of terminal leaves and stem tips, coupled with increase in soluble nitrogen in these organs shortly following the transfer from 65° to 95°.

In the consideration of nitrate assimilation in peach, it was stated that apple and peach (of the varieties employed) were apparently similar in their ability to synthesize organic nitrogen at the respective sand temperatures. The data of table II would seem to support this statement. Added nitrate resulted in some increase in concentration of organic nitrogen at each temperature, and taking current roots and old roots together (tables V and VI) this increase was greatest at 65°, lower at the two extremes of temperature, and otherwise followed closely in trend the curve for absolute reducase activity in apple (fig. 11). So also did the increase in amount of top growth (fig. 11, table II) which resulted from added nitrate as well as the absolute amount and percentage of organic nitrogen in the tops, analyses of which are not reported in detail.

At 65° and lower, added nitrate was definitely associated with increase in amount of current root growth (table II). In fact at 45° no new roots developed on the series lacking nitrogen in the culture medium. There were many root primordia present in both series, however, although in the latter group they failed, during the period of these experiments, to emerge through the periderm sufficiently to be readily noticeable macroscopically. A reason for this effect of added nitrate was not established, but assimilation of nitrate makes available materials which are essential for the development of new tissues such as amino acids and other relatively simple soluble forms of organic nitrogen. In the lack of new synthesis from inorganic nitrogenous nutrients, amino acids or related compounds are derived solely from reserves stored in initially present tissues. The assimilation of nitrate thus furnishes an additional source of organic nitrogen which conceivably might result in greater root growth, particularly at lower temperatures when there were present carbohydrates in abundance (table V).

Whereas at lower temperatures the yield of current roots was increased by added nitrate, the same nutrient treatment at 75° and higher was associated with a decrease in amount of roots produced as compared with comparable cultures lacking nitrate. In fact at 90° many of the current roots died following application of the complete nutrient solution, but persisted in case of the cultures grown continually with no external source of nitrogen. Applications of nitrate in cases where it was assimilated have frequently resulted in lessened growth and even in death of the plants when available carbohydrates were limited in amount, as is frequently the case at relatively high temperatures (**12, 18**). In the reduction and assimilation of nitrate there necessarily occurs oxidation of carbohydrates or their derivatives, resulting in a decrease of those present unless supplied by new synthesis. In the current roots at 75° and higher, carbohydrates were definitely lower than in comparable trees that were grown with no external source of nitrogen (table V). With assimilation of nitrate at 90°, available carbohydrates became practically exhausted and death of the roots occurred.

Without knowledge of the anatomy of the root systems concerned, the results on concentration and quality of carbohydrates in the current roots would seem impossible of accurate interpretation. Of course, as already explained, added nitrate resulted in a decrease in percentage of carbohydrates, but the several analyses of apple and peach were remarkably consistent in showing that with a given nutrient treatment sugars were highest at low temperatures (tables III, V), and that there was directly correlated with increase in temperature a marked decrease in concentration of sugars. These results seem reasonable enough in view of the fact that the rate of respiration of the current roots was undoubtedly less at lower than at high temperatures. At least this was found to be true in the aerial organs of peach by actual measurement of the rate of carbon dioxide exchange (**23**), and the pH of the root tissues as already recorded was in harmony with this view. As will be shown later, however, differences in rate of respiration did not furnish a complete explanation for the accumulation of sugars at low temperature.

In contrast, starch was relatively low in the current roots of both genera at 45°, 50°, and 55°. This does not mean that condensation to starch cannot occur at these temperatures, but rather that the roots continually under those conditions had produced comparatively little potential starch storing tissue. The juvenile cortex which contributed in such large part to the samples as analyzed at low temperatures was apparently not a starch storing tissue. Only occasional small grains of starch were noticed in the cortex, none in the extensive embryonic zone, and few in the relatively immature stele. On the other hand, when some of the trees of both genera were shifted from 60° to 45°, starch accumulated in the current roots in much higher concentration than in any other groups of cultures. But the roots at 60° exhibited at the time of shift much more secondary phloem and xylem parenchyma, both potential starch storing tissues, than was observed in the roots continually at lower temperatures. At 45° starch as well as sugars has frequently been found to accumulate in the current roots of apple and peach when there were initially present potential starch storing tissues (**19, 22, 23**).

At temperatures of 60°, 65°, and 75° starch was found in moderate concentration (tables III, V) in the current roots of both genera, but as already mentioned there was present a considerable amount of potential starch storing tissue. Sugars on the other hand were low, as expressed on the basis of the percentage present in the entire current root system, and most of the reducing sugars and sucrose were found principally, not in the older secondary tissue, but in relatively meristematic elements in the vicinity of the cambium and embryonic zone of the root tips. The concentration of sugars in such cells did not appear to be much, if any, lower than at 50° or 55°, but the proportionate amount of meristematic tissue notably high in sugars was much greater at low than at higher temperatures (figs. 6–9). Hence the gross percentage data as it appears in tables III and V might well give an erroneous impression in the lack of anatomical evidence.

There was little meristematic tissue at 85° and 90° (fig. 9), which, coupled with an undoubtedly high rate of respiration (in cells containing respirable material), contributed to the very low percentage

of sugars as given for apple and peach in tables III and V respectively. The rather high percentage of starch in the current roots of peach at 85° is in contrast to apple where starch was relatively low. However, microchemical observations of the former genus were in accord with the results of macroanalysis. Starch grains persisted in the mature secondary parenchymatous elements of phloem and xylem long after sugars could be detected only in and near the embryonic zone of the root tip and in the very meager cambium region (fig. 9). It appears not improbable that enzymatic hydrolysis of starch was adversely affected at 85° in peach, although as will be shown presently this was seemingly not true in the old initially present roots. In any event, as the tissues of the current roots differentiated there was complete loss or disorganization of the more or less optically opaque protoplasm, and there was left the residue of starch as indicated in table V. This point will be discussed further.

As might be anticipated from the anatomical change already recorded, the shift from 60° to 95° resulted in a marked drop in sugars in the current roots of both genera. Accompanying this decrease and loss in amount of meristematic tissue there was an increase in rate of differentiation, with concomitant lignification and development of fibers, necessarily at the expense of sugars or their derivatives since cell walls in their various forms are condensation products of the simpler carbohydrates. Closely associated with these changes there was loss of starch, the hydrolysis of which appeared to continue in the respective cells as long as there could be detected any protoplasm. However, even after sugars were practically exhausted, presumably through respiration and contribution to cell walls (and in some degree through nitrate assimilation where nitrate was present), starch grains were still left undigested in cells apparently inert, although not always devoid of all other contents.

Following the shift just mentioned, there were left in the cortex and in the secondary parenchymatous tissues of the stele occasional cells containing materials other than and in addition to starch. These cells were present singly or in groups and were as a rule surrounded by optically empty tissue. The contents of such cells appeared more or less granular, had a pH value of 4.4–4.2, usually con-

tained many calcium oxalate crystals, and gave positive reactions for proteins, various nutrient elements, and tannins. Protoplasm of vigorous meristematic tissues did not give positive reactions to the usual protein reagents without preliminary denaturation, but the contents of the cells referred to reacted to most protein tests without preliminary treatment. It would seem, therefore, that a temperature of 95° resulted in disorganization of the protoplast of occasional cells whose contents (with the possible exception of sugars) remained unavailable to developing tissues of the current roots. This occurred in both apple and peach.

Cells of similar description were present at 90° and 85° but were noticed chiefly in the cortex as it began to turn brown. Such cells remained as described even after death of the cortex. At 75° cortical tissue occasionally exhibited cells of this type, whereas such cells were absent at the lower temperatures employed.

Little need be said concerning the percentage dry matter in the current roots of apple and peach (tables III, V). Proceeding from low to high temperature there was a consistent increase in such percentage. That this was not due in any considerable part to accumulation of sugars and starch would seem to have been made sufficiently clear. It was undoubtedly due principally to the definitely higher proportion of lignified xylem and mechanical elements which occurred with increase in temperature (figs. 6–9) and which necessarily developed through condensation of sugars or their derivative.

The concentration of carbohydrates in the old initially present roots of apple and peach may be mentioned briefly (tables IV, VI). The percentage of sugars showed considerable fluctuation, but in general it was found to be highest at lower temperatures, presumably because there was less vigorous respiration and because less carbohydrate material was required for the limited growth of roots that occurred at 45°, 50°, and 55°.

The figures for starch and dry matter in the initially present roots are also similar in trend to those for sugars, with one significant exception. The lowest percentage of both starch and of dry matter in the old roots of apple and peach was at 85°, not at 90° or 95°. At the two higher temperatures, as verified by repeated microscopic observation, starch was present in high concentration in

parenchymatous tissues. Obviously under the conditions of these experiments the temperatures of 90° and 95° seriously limited digestion of starch in the old roots.

The tops of the apple and peach trees were not analyzed macrochemically for carbohydrates, but during the two months' period of these experiments there was found to be an abundance of sugars and starch in the old stems in all cases. This was likewise true for the current top growth with the following exceptions: at 45°, 50°, 90°, 95°, and occasionally at 85°, slight wilting of leaves sometimes occurred during the middle of the day. This apparent lack of water was undoubtedly associated with the meager development of current roots at the temperatures mentioned. Although it has already been stated that these trees took in considerable water through their old roots, the photosynthetic activity of the trees was seemingly limited. At least the leaves and current stems were relatively low in sugars and starch.

## Summary

Young Stayman apple trees and similar Elberta peach trees were obtained from a commercial nursery in the fall of 1933. The tops were pruned back severely, all small roots were removed, the trees were planted in white quartz sand in self-draining culture jars and held in storage at 35° F. until January 31. During that period the sand was kept moist with tap water and by the weekly application of a nutrient solution complete except for nitrogen. On January 31 when experimental treatments commenced the buds had not expanded noticeably and no new roots were present.

For the following experimental period of two months the trees were grown in the original containers without transplanting, but were shifted to water baths so that some of the trees of each group were held continuously at sand temperatures of 45°, 50°, 55°, 60°, 65°, 75°, 85°, 90°, and 95° F. respectively. The air temperature was in all cases about 60° to 65° at night and 65° to 70° during the day. Temperatures mentioned in the following paragraphs refer to the temperature of the sand in which the roots were grown.

1. In both apple and peach the maximum yield of current roots and of current tops occurred at a sand temperature of 65°, and with increase or decrease in the temperatures employed there was a de-

crease in yield of roots and aerial organs. At 45° root primordia were present but few roots emerged through the periderm during the period of these experiments. At 95° no new roots appeared and the old roots eventually died.

2. At 65° and lower temperatures the newly developed roots of both genera were typically white, of relatively large diameter, extremely succulent, lacking in mechanical strength, and they characteristically exhibited few fine laterals.

3. In contrast, at 75° in both genera the cortex turned brown and gradually sloughed off. The remainder of the root, the central cylinder, was typically very woody, of considerable mechanical strength, and lacking in succulence. There were present many fine lateral roots. At 85° and 90° the roots were similar in quality but much smaller in diameter and less extensive.

4. The initially present pruning wounds of the old roots of both apple and peach callused over earliest at 85° and 90°. At the latter temperature there was often incomplete covering of the pruning wounds by callus tissue and the callus became completely suberized when only a few cells thick, cell division having ceased. There was in general at 85° complete covering of the pruning wounds by a rather thin layer of callus which suberized and turned brown rapidly. At 75° wound callus tissue was thicker and remained white for a somewhat longer time. At 65° and lower the callus tissue developed more slowly, failed to mature, was white and fluffy in appearance, and broke off easily in handling. More or less satisfactory callusing occurred at 85°, therefore, a temperature at least 10° higher than that which could be considered favorable for root growth under the conditions of these experiments.

5. In both genera at 65° and lower there was present directly back of the root tip an extensive zone of embryonic tissue, the cortex persisted for the duration of these experiments, the pericycle although exhibiting considerable cell division did not become suberized externally, nor were there produced any pericycle or phloem fibers. At several centimeters from the root tip there was also present in transverse section a wide band of undifferentiated tissue in the region of the stelar cambium, but development of secondary tissues and lignification of xylem were notably slow. These factors were therefore as-

sociated with the general condition of succulency and lack of mechanical strength.

6. At 75°, in case of both apple and peach, the embryonic zone of the root tip was much less extensive than at lower temperatures, owing to relatively early differentiation of tissues which was soon followed by the development of secondary elements and death of the cortex. Accompanying maturation of the juvenile cortex there was developed an active cork cambium that produced externally cells that suberized rapidly, and internally parenchymatous cells and many heavy walled fibers. The stelar cambium was likewise active and secondary phloem and xylem were extensive. Conspicuous elements of the phloem were the large bundles of strongly developed fibers, and, with the exception of ray cells, the entire xylem was thick walled and lignified. These factors were obviously associated with the general lack of succulence and considerable mechanical strength of the roots concerned.

7. At 85° and 90° the primary tissues were made up of a comparatively small number of cells and differentiation occurred even earlier in the roots of apple and peach than at 75°. The embryonic zone of the root tip was therefore extremely limited in volume. The cortex died early and the pericycle produced externally a few cells that suberized rapidly, although frequently the periderm was non-continuous especially at the higher temperature. There were present a few small fibers in pericycle and phloem, but the amount of phloem and xylem was limited, first by the relatively small number of cells in the meristem of the root tip and second by a stelar cambium region only two or three cells wide in transverse section. In fact mature phloem cells and strongly lignified xylem elements frequently abutted one upon the other. These factors therefore contributed to the small diameter of the roots concerned and to their extreme lack of succulence.

8. Respective differentiated tissues of the current roots of the two genera were not noticeably different in H ion concentration under comparable conditions. Young cortical cells were approximately pH 5.2–4.8, but with rapid maturity of the cortex at temperatures of 75° and higher the cells became more acid (4.4–4.2). Cellulose walls apparently failed to react with the usual indicators. Lignified walls

gave a so-called pH value of 4.4–4.0. At 75° and higher a relatively large percentage of the current root system consisted of lignified xylem elements and fibers that invariably exhibited the acid reaction mentioned. The cell contents of phloem were much less acid. Recently developed cells had a pH value of 6.2–5.9, mature cells 5.2–4.8, and the phloem matured most rapidly at higher temperatures.

Proceeding from low to high temperatures the embryonic tissues of cambium and root tip became increasingly acid in reaction. This was apparently associated with relatively rapid respiration and accumulation of carbon dioxide in the tissues concerned. The real pH of meristematic tissue was therefore problematical, but the pH was under all conditions much less acid than that of mature xylem walls and seemed to approach the more nearly alkaline condition of young phloem cells (6.2–5.9) at temperatures of 65° and lower. On the other hand, at 90° the pH of meristematic tissue was probably about 5.0–4.8. The rapidly maturing non-succulent roots at temperatures of 75° and higher were therefore low in proportion of less acid tissues.

9. Nitrate was freely *absorbed* by the current roots of apple and peach at the respective temperatures employed, and without exception was limited exclusively to these organs for the duration of the experiments. Whereas the temperature of the nutrient medium affected little, if at all, the ability of the roots to absorb nitrate (provided they were not already saturated to capacity with it), temperature had a very marked influence on the ability of the roots to reduce nitrate to nitrite, ammonium, and amino acids. This process of reduction and synthesis with accompanying oxidation of carbohydrates or their derivatives may be included in the term *nitrate assimilation.*

10. ECKERSON'S (**6**) method was employed for determining the *reducase* value or comparative nitrate reducing ability of aqueous extracts of apple root tissue. Chemical analyses indicated that reducase activity furnished an index of the relative rate of assimilation of nitrate. Certain samples that consisted principally of the relatively meristematic tissue of the root tips were tested for reducase. Proceeding from low to high temperature the results showed that there was a consistent increase in rate of nitrate reduction. But at

higher temperatures there was present relatively little meristematic tissue and it was comparatively more acid than at lower temperatures, a fact in itself unfavorable for reducase activity. At 65° and lower the intensity of nitrate reduction in the root tips was much less than at higher temperatures, but samples even from the proximal or oldest end of the current roots at 65° contained a fairly high proportion of active cells and exhibited about the same degree of reducase activity as the tips.

Coupled with this high proportion of active, less acid tissues and a relatively low percentage of inert, more acid elements such as mechanical fibers, lignified xylem, and strongly suberized periderm, there was produced at 65° the greatest yield of roots and aerial organs. Consequently it seemed logical to find in the current roots of the apple trees at 65° the highest absolute reduction of nitrate as computed on the basis of reducase activity. It should be recalled, however, that these roots were lacking in mechanical strength.

11. Macro- and microchemical analyses of apple trees corroborated the statements in the foregoing paragraph, and similar analyses of peach trees before and after addition of nitrate indicated that assimilation of nitrate was essentially similar in both genera at the respective temperatures employed. With reduction of nitrate there necessarily occurs oxidation of carbohydrates or their derivatives. Analyses ultimately showed that where nitrate was reduced carbohydrates were lower than in comparable cultures which received no external source of nitrogen.

12. At 65° and lower the current roots of both apple and peach were notably high in percentage of organic nitrogen. This was intimately associated with the relatively large proportion of meristematic tissue present at these temperatures. At higher temperatures there was proportionately less such tissue and the concentration of nitrate-free nitrogen was extremely low.

13. The trees of both genera which were grown continually at 55° or lower were high in percentage of reducing sugars and sucrose but low in starch. However, the roots of these trees were high in proportion of comparatively meristematic tissue that contained sugars but did not store starch. Likewise the persistent juvenile cortex which was not a potential starch storing tissue contributed in

large part to the aliquots analyzed macrochemically. On the other hand, when some of the trees of both genera were shifted from 60° to 45° starch accumulated in the current roots of both groups in much higher concentration than in any other case, but the roots at 60° exhibited at the time of shift much more secondary phloem and xylem parenchyma, both potential starch storing tissues.

14. At 75° and higher the percentage dry matter in the current roots of both genera was high. This was intimately associated with the presence of thick walled lignified xylem and mechanical fibers. Sugars and starch were low, probably in part because of a comparatively high rate of respiration, but the development of thick walled fibers and strongly lignified xylem walls was necessarily at the expense of sugars or their derivatives, as cell walls in their various forms are condensation products of carbohydrates.

15. The current roots of both genera were extremely deficient in sugars and starch at 85° and 90°. The old initially present roots of the trees at 90° and 95° contained, however, a very high concentration of starch. This temperature apparently seriously limited digestion of starch in the old roots.

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# MORPHOLOGY AND BIOLOGY OF SOME SPECIES OF ODONTIA

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(WITH NINETEEN FIGURES)

## Introduction

The Hydnaceae, as recognized by most students of the group, is a family of the Hymenomycetes containing a heterogenous group of pileate, sessile, reflexed, and resupinate plants, characterized by the possession of teeth or spines, granules, and warty protuberances which usually point downward. These last are covered by a hymenium which usually does not extend over their apices.

Considerable intergradation occurs within the family. As a result much confusion in classification has arisen because of the difference in emphasis placed on the various diagnostic characters by different students or even by the same investigator in the course of his studies.

In 1929 the late Professor C. H. KAUFFMAN suggested the desirability of a cultural study of this group. The classification of the species collected for this study necessitated a review of the whole taxonomic situation and a comparative study of the morphology of the species. A start has been made in the study of the biology of the resupinate species of the Hydnaceae. Eleven species have been grown and their development compared on synthetic media. It is hoped that the information thus obtained will help to throw light on the relationships in the group and lead to a more usable and stable classification.

Although much attention has been devoted to the taxonomy of the Hydnaceae, comparatively slight attention has been given to a study of them in pure culture. Difficulty has been experienced in germinating the basidiospores of species in the Hydnaceae. When germination was secured, the cultures usually remained sterile or produced structures which were described as bulbils, oidia, or chlamydospores. In only a few instances were basidiospores obtained.

BREFELD (6) was the first to study species of this family in culture.

He reported difficulty in securing germination of basidiospores in the genera *Sistotrema*, *Hericium*, *Hydnum*, *Mucronella*, *Odontia*, and in the species *Grandinia granulosa* Fr. He succeeded in germinating the spores of *Kneiffia setigera* Fr., *Grandinia crustosa* (Pers.) Fr., and *G. mucida* Fr. His cultures of these all remained sterile. The basidiospores of *Phlebia merismoides* Fr., *Ph. radiata* Fr., *Ph. contorta* Fr., and *Ph. vaga* Fr. were easy to germinate and oidia were produced in eight days. The spores of *Radulum orbiculare* Fr., *R. pendulum* Fr., *R. fagineum* (Pers.) Fr., *R. molare* Fr., and *R. lateum* Fr. germinated easily. The side branches of the mycelium became constricted into beadlike chains which he did not consider typical conidia. Only *R. pendulum*, now believed to be a tuberculate form of *Corticium subcostatum* Karst., produced basidiospores in his cultures.

FALCK (17) reported fruiting of *Phlebia merismoides* in cultures, not only from basidiospores but also from a single oidium.

LYMAN (30) studied a great number of Hymenomycetes, especially species in the Thelephoraceae. He apparently also studied many of the Hydnaceae but unfortunately was unable to give much information concerning this group because he lost his data. He reported, however, that chlamydospores were obtained for this family. HOTSON (23) studied the production of bulbils and similar propagative bodies in the Hymenomycetes. He found bulbils, oidia, and basidia in cultures of *Grandinia crustosa* (Pers.) Fr., now *Odontia crustosa* (Pers.) Quél.

DESEYNES (15) and PATOUILLARD (36) observed macro- and micro-conidia in collections of *Hydnum erinaceus* Bull. and *H. coralloides* (Scop.) Fr. They mentioned the resemblance of micro-conidia to basidiospores, which, as will be shown later, they probably were.

BROOKS (7) cultured *Hydnum coralloides* on ash and secured the formation of small but typical fructifications in about four months' time. Further cultures produced abnormal fruit bodies with rudimentary spines. He secured this fruiting from spores which were collected on a sheet of clean glass and transferred to tubes containing the wood.

## Methods of study

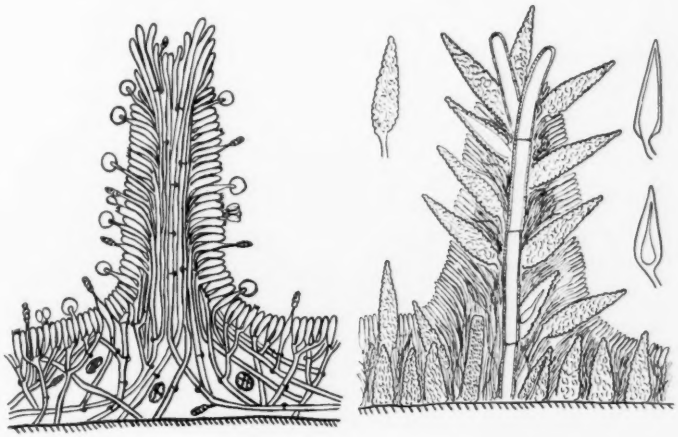
BURT (8–11) suggested a standard method of procedure for the study of specimens of the Thelephoraceae. Briefly this consists in

moistening a bit of the hymenium with alcohol, wetting it with water in order to section the material easily, then transferring the sections to 7 per cent potassium hydroxide solution to expand the tissue to nearly natural size. In some instances lactic acid was used because potassium hydroxide darkened the sections. Permanent slides were made by staining the sections with water soluble eosin, then mounting them in dilute glycerin and, when ready, cementing the coverglasses to the slides. This is essentially the procedure followed by OVERHOLTS (**35**). He acidified the eosin stained sections with acetic acid so that the color would not fade so readily. BOURDOT and GALZIN (**5**) and DONK (**16**) used potassium carbonate solutions colored with eosin, and chloral hydrate with iodine in their investigations of the Hymenomycetes.

In recent years several European investigators have studied the effects of various reagents on fungi, with the result that the reactions have been utilized by some as important diagnostic characters. BOURDOT and GALZIN (**5**) found that the use of potassium carbonate with eosin stained the protoplasts of certain types of mycelia more readily than others and used this as a supplementary character in delimiting species. Gloeocystidia are also more easily detected by the use of potassium carbonate with eosin or chloral hydrate with iodine. KÜHNER (**27**) has even gone so far as to use such reactions as a means of distinguishing genera. He maintains that the morphological characters alone are insufficient to separate the genera *Lentinellus* Fayod and *Lentinus* Fr., and has emended the former by recognizing the amyloid spore as the principal diagnostic character. In the Hydnaceae, the genus *Dryodon* (*Hericium*) is also characterized in part by the amyloid spore character. A striking reaction is noted by BOURDOT and GALZIN (**5**) in *Odontia* (*Acia*) *uda* (Fr.) Bres., which turns purple when exposed to the fumes of ammonia. They consider this a reliable means for separating this species from *O.* (*Acia*) *fusco-atra* (Fr.) Bres.

Different workers have used reagents of various kinds and strengths. The present studies show the necessity for giving the strengths of reagents used. For instance, a careful examination of sections from fresh specimens of *Odontia arguta* (L.) Quél. shows small capitate structures, herein called "capitate cystidia," which at

first may easily be mistaken for air bubbles or drops of oil in sections which have not been fully expanded or which contain air (fig. 1). Once this structure is recognized, it is easily distinguished. However, Amann's mounting medium, chloral hydrate with iodine, 95 per cent alcohol, and 0.5 per cent potassium hydroxide all rapidly dissolve the globose cap from the capitate cystidium, leaving a small, cylindrical, hyphal stalk. Although 5 per cent potassium carbonate also dissolves the cap, it acts more slowly.



FIGS. 1, 2.—Fig. 1 (left), diagrammatic sketch showing structures of spine, axillary hyphae, and capitate and oxalate incrusted cystidia. Fig. 2 (right), same showing structure of spine, incrusted axillary hyphae, imbedded and projecting cystidia. Sketch at right of spine shows cystidia after incrustations were dissolved.

Inasmuch as several of the common reagents will alter certain of the diagnostic characters, it is imperative that at least a preliminary examination of the material be made in distilled water and that the reagents be then added by drawing them under the coverglass with a piece of filter paper while the mount is under observation.

Studies of dried specimens, following the procedure outlined by BURT (**8–11**) or OVERHOLTS (**35**), may sometimes fail to reveal the presence of diagnostic characters, as 7 per cent potassium hydroxide and 95 per cent alcohol have been found too strong, destroying or

obscuring certain types of structures found in Basidiomycetes. This may account for some of the discrepancies for the same species between BURT's descriptions and those of some European workers, especially with reference to the presence or absence of incrustations on the hyphae and cystidia.

The following technique has been employed in studying specimens. Sections were mounted in distilled water for an examination for the presence of cystidia, cystidium-like organs, and incrustations. They were then treated either with 0.5 per cent potassium hydroxide or with 5 per cent potassium carbonate colored with eosin to expand and stain the spores and tissues. In the case of material which did not section readily, a small piece was soaked in water for half an hour or longer, until it sectioned easily.

Chloral hydrate with iodine, as used by STEVENS (**39**), was found to be a satisfactory reagent to follow distilled water when a detailed study of the section was wanted, inasmuch as it expands the tissues better than either potassium hydroxide or potassium carbonate. However, it has some solvent action. The iodine in this solution gives a yellow-brown color to the hyphae and a darker brown color to the gloeocystidia. Amyloid spores of course turn blue. Sometimes the definition of an object expanded with this reagent is unsatisfactory, and in those cases the 5 per cent potassium carbonate with 0.01 per cent eosin was used. This dye stains the hyphae so that a sharp definition can be secured. The use of Amann's mounting medium or 5 per cent potassium carbonate with various dyes such as cotton blue or eosin has been helpful in distinguishing individual hyphae. As yet no one reagent has been found which is entirely satisfactory for the study of all specimens.

A solution of 10 per cent hydrochloric acid was used to remove the calcium oxalate, and with this obstructing material dissolved the structure of the specimens can be more readily ascertained.

It is difficult to make tissue cultures of the resupinate Hydnaceae, except in the case of a few species which have rhizomorphs, because the subiculum and teeth are thin. Only one tissue culture was secured. *Odontia fragilissima* (B. & C.) Brown was cultured from a rhizomorph after the usual procedure of surface sterilization in mercuric chloride followed by several changes in distilled water.

Specimens were collected in the field and wrapped in waxed paper. A spore fall was obtained by placing a specimen over sterile petri dishes or capsules. When the deposit of spores was visible (after 2–12 hours) sterile distilled water was added. The proper dilution was determined by making a fine spray on a glass slide and examining individual drops to see that the number of spores did not exceed two or three per drop. The spore suspension was sprayed on previously poured agar plates as described by KAUFFMAN (**24**). After germination, which takes 12–72 hours at a room temperature of approximately 20° C., germinated single spores were isolated by cutting out small blocks of agar 1 mm. square under the low power of the microscope. The spore was transferred to a sterile agar plate or to a slanted test-tube. Difficulty similar to that reported by BREFELD (**6**) was experienced in securing the germination of spores. Spores from field material germinated in only 17 instances out of 155 attempts. Germination from basidiospores produced in culture was much more certain.

Experiments were conducted using a maltose agar,[1] LEONIAN'S agar (**28**), KOTILA'S agar (**26**), a 6 per cent oatmeal agar, and a 2 per cent malt extract agar. KOTILA'S agar was found to be the most satisfactory for vegetative growth and fruiting, inasmuch as basidiospores formed on this substratum 5–10 days sooner than on the others. Therefore this was used as the standard medium on which these fungi were grown. KOTILA'S agar did not always solidify after the usual sterilization at 15 pounds' pressure for 15 minutes in the autoclave, and this difficulty was overcome by increasing the quantity of the agar from 20 to 30 gm. per liter of water or by sterilizing the agar at 10 pounds for 20 minutes.

The various species were also grown on filter paper pads saturated with the different nutrient solutions used for these agars. Filter paper was cut and folded into U-shaped pads and placed in an inverted position in small glass capsules, then dry-air sterilized.

Some of the species studied readily formed a hymenium in about a month after isolation of the single spores. Other species would not

[1] KAUFFMAN'S maltose agar—to the liter: maltose 5.0 gm., peptone 0.1 gm., magnesium sulphate 0.5 gm., potassium di-hydrogen phosphate 0.25 gm., calcium nitrate 0.1 gm., and agar 15 gm.

fruit readily in culture, and in these instances, fruiting could sometimes be brought about by the following technique. The fungi were grown in glass capsules on filter paper pads, moistened with different nutrient solutions. After sufficient mycelium was formed (10 days or more), the nutrient solution was replaced with sterile distilled water. Fruiting occurred 10–20 days later, depending upon the species.

Sterilized thin slabs of white pine, basswood, and elm were also used in glass capsules with a nutrient solution, or with their respective wood decoctions.

Approximately 600 collections of resupinate Basidiomycetes were made. Many of these were sent to European workers for identification. ABBÉ H. BOURDOT and M. A. DONK both have identified specimens, and have given many helpful comments, this assistance being of great value. In the following discussion, the citation of the author's collection number indicates that a duplicate of that specimen has been deposited in the Herbarium of the University of Michigan.

The following species were studied and cultures were secured from those marked with an asterisk. All the specimens cultured were collected in the vicinity of Ann Arbor, Michigan, with the exception of *Odontia bicolor* from Louisiana.

* *O. arguta* (Fr.) Quél.
* *O. fusco-atra* (Fr.) Bres. (three collections)
* *O. uda* (Fr.) Bres.
* *O. stenodon* (Pers.) Bres.
* *O. brinkmanni* Bres.
* *O. hydnoides* (C. & M.) v. Höhn.
* *O. bicolor* (A. & S.) Bres. (from Louisiana)
* *O. fragilissima* (B. & C.) Brown
* *O. separans* (Peck) Brown
*O. himantia* (Schw.) Bres.
*O. fimbriata* Fr.
*O. setigera* (Fr.) Miller

## Results

*Odontia arguta* (figs. 1, 3, 4, 5, 6, 7)

*Odontia arguta* (Fr.) Quél.,–Fl. Myc. Fr. 435. 1888.
BRESADOLA, Atti. Accad. Rovereto III. **3**:98. 1897.
BOURDOT & GALZIN, Hym. de France p. 427.

Fructification effused in patches up to 60 cm. long by 30 cm. wide, usually smaller, soft, arachnoid to tomentose, thin, adnate, cracking on drying, white to colonial buff (MP)[2] when fresh, darkening slightly when dry; margin indeterminate, pubescent; spines concolorous with the hymenium, tips lighter, granulate to cylindrical subulate, sometimes slightly flattened and connected at base, crowded, 1–3 mm. long, apices penicillate; hyphae thin walled, 2–4 $\mu$ in diameter, loose in subiculum, more compact under the hymenium and in axes of the spines, clamp connections present; basidia 10–16×3–5 $\mu$; spores hyaline, smooth, variable from broadly oval to subglobose, often apiculate, 3–5 (6)×3–4.5 $\mu$; capitate cystidia arising from the basidial layer or from tramal hyphae, present on sides and between the spines, not in subiculum, the cap part 7–9 $\mu$ in diameter on a hypha 2.5–3 $\mu$ in diameter, cap soluble in alcohol, potassium hydroxide, chloral hydrate with iodine, hot water, and slowly so in potassium carbonate; the second type of cystidia (fig. 1) of two forms present in the subiculum and on the sides and apices of the spines: the first 1–2 $\mu$ in diameter, the second 3–5 $\mu$ in diameter, constricted near apex to 2 $\mu$ in diameter, the apices of both incrusted with calcium oxalate 2.5–3.5 $\mu$ in diameter; masses of calcium oxalate or other mineral matter soluble in hydrochloric acid often found in the subiculum.

This is by far the most common species of *Odontia* in the vicinity of Ann Arbor. It is found on the under sides of rotten logs of both deciduous and coniferous trees. In addition to specimens from Michigan, material from Canada, Iowa, New York, Pennsylvania, and

[2] The technical color names indicated by (MP) are from MAERZ & PAUL (**31**). Names followed by (R) are from RIDGWAY (**38**). The writer has used the recent publication of MAERZ & PAUL because RIDGWAY's Color Standards and Nomenclature is out of print. Many of the names used by RIDGWAY are retained by MAERZ & PAUL, who also included in the index a definite reference to most of the RIDGWAY color names.

North Carolina has been studied, which indicates that it has a wide distribution in North America.

One of 15 attempts to culture this species was successful. The basidiospores germinated on agar plates in 3–5 days at room temperature. They produced a single germ tube which became branched within 48 hours. The mycelium was mostly submerged in the agar. On the surface it developed a thin compact film. The color varied from white to cream, and when fruiting, it became cream colored. Spores were produced after a period of 20–30 days. They germinated somewhat readily at the end of 3 days.

This fungus produced spines on nutrient agar (fig. 3), on filter paper pads (fig. 4), and on wood (figs. 5, 7). Nearly typical spines were produced on the under side of the filter paper pads (fig. 4), and branched upright structures were formed on the upper surface of filter paper pads, on agar, and on wood. The capitate cystidia and the oxalate incrusted cystidia, so characteristic of this species in nature, were not produced in culture.

Cultures grown in light or in darkness at room temperature did not show any appreciable variation in the rate of growth or fruiting.

This species is homothallic, as shown by the fact that 22 monosporous cultures produced teeth with hymenium, typical basidia, and basidiospores. Clamp connections were present on all mycelia.

*Odontia fusco-atra* (figs. 8, 9, 10)

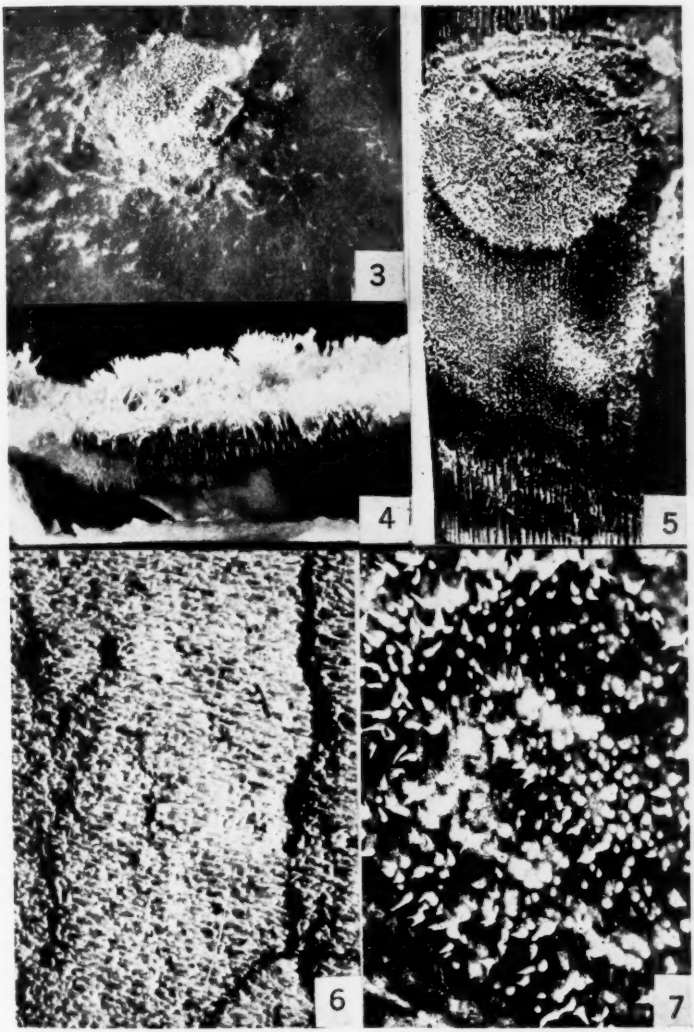
*Odontia fusco-atra* (Fr.) Bres.,–Atti. Accad. Rovereto III. **3**:95. 1897.

*Acia fusco-atra* (Fr.) Pat.,–BOURDOT & GALZIN, Hym. de France p. 417.

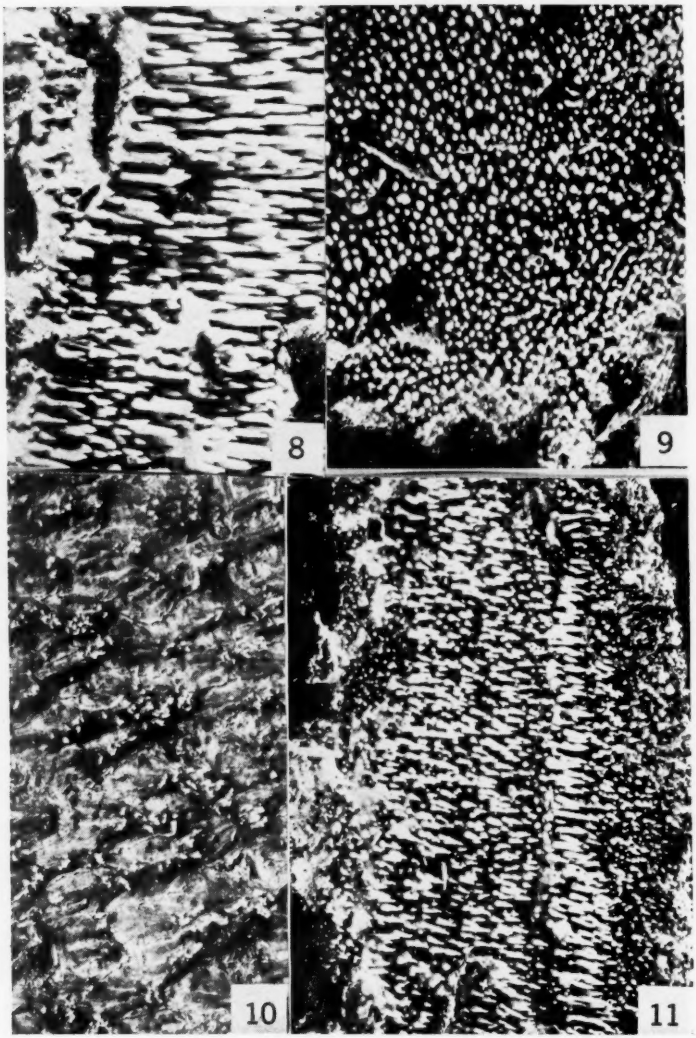
*Mycoacia fusco-atra* (Fr.) Donk,–Med. Ned. Myc. Ver. **18–20**:152. 1931.

*Hydnum carbonarium* Peck,–N.Y. State Mus. Rept. 40. p. 55. 1887.

Fructification effused in patches up to 60×10–20 cm., thin, adnate, color variable from bluish white to peach beige (MP) to cinnamon brown (MP) to burnt umber (MP) when fresh, art brown (MP) to black when dry; margin bluish white to yellowish, pruinose or finely byssoid; spines slender, subulate, pointed or with apices di-




FIGS. 3–7.—*Odontia arguta:* Fig. 3, culture in petri dish on KOTILA'S agar; natural size. Fig. 4, same on filter paper pad showing typical teeth produced on lower surface; ×2. Fig. 5, same on pine wood; natural size. Fig. 6, fruit body (no. 426) on rotten hardwood. Ann Arbor, Mich.; ×5. Fig. 7, culture showing detail of spines; ×5.




FIGS. 8–11.—Fig. 8, fruit body of *O. fusco-atra* (no. 417) side view, on basswood. Collected near Ann Arbor, Mich. Fig. 9, looking down on tips of spines of same fructification. Fig. 10, dried specimen of same showing clumping of spines or "molariform" teeth. Photograph by EDGERTON. Fig. 11, *O. uda* (no. 426) collected near Whitmore Lake, Michigan. ×5.

vided, concolorous with the subiculum or with tips lighter colored; subiculum hyphae moderately dense, becoming more compact just under the hymenium, 2.5–3 μ in diameter, thin walled, with clamp connections; hyphae in axes of spines compact, parallel, ascending to somewhat interwoven; cystidia incrusted, 5–8 μ in diameter, abundant in axes of the spines, usually imbedded, sometimes projecting from the spines at various angles; cystidioles occasionally present on the sides of spines, subulate, difficult to detect; basidia 14–20×3–4 μ; spores oblong cylindrical, sometimes slightly curved, 4–5×2 μ.

Twelve collections from the vicinity of Ann Arbor (nos. 417, 425, 81) were studied and also one collection each from New York, Ohio, North Carolina, and Holland respectively. The New York collection (no. 34) was determined by BOURDOT.

The variations noted in the color of this species, when compared with the relative constancy of color in a number of other species of the group, suggested that two or more species might be involved. However, a study of two fresh collections showed a change in color from bluish white to cinnamon brown (MP) in less than an hour. The burnt umber (MP) color appeared in about two hours, and the dried collections varied as noted. Material has been collected in the field in which the specimens were uniformly cinnamon brown (MP) and uniformly burnt umber (MP). Other collections have shown a mottled appearance caused by transitional changes in different areas.

Fourteen monosporous cultures were made successfully from three specimens collected in 1929, 1930, and 1932. Monosporous cultures of these had clamp connections, produced basidia and basidiospores. Repeated isolations behaved likewise, thus establishing the homothallic nature of the fungus. A variation in the time it took monosporous cultures to fruit is worthy of mention. Fruiting usually occurred in 30 days at room temperature on maltose agar and in 20 days on KOTILA'S agar. Some cultures, however, required three to four months to mature.

This species produced mostly submerged mycelium with merely a scant floccose film over the surface of the agar. The fructification was a yellowish crust on the surface of the agar and was composed of a compact palisade-like layer of basidia with here and there an up-

right incrusted hypha. These incrusted hyphae or cystidia were similar to those found in the axes of the spines, but differed in having larger granules forming the incrustation, and they usually occurred solitary. The striking color change exhibited by field collections was absent from cultures on agar or on filter paper pads.

An examination of the type of *Hydnum carbonarium* in the Herbarium of the New York State Museum resulted in finding that the specimen is a depauperate or young stage of *Odontia fusco-atra*.

*Odontia uda* (fig. 11)

*Odontia uda* (Fr.) Bres.,–Atti Accad. Rovereto III. **3**:97. 1897.

*Acia uda* (Fr.) B. & G., –Hym. de France p. 414.

*Mycoacia uda* (Fr.) Donk,–Med. Ned. Myc. Ver. **18–20**:151. 1931.

Fructification effused, in small patches, 3–6×1.5–2 cm., thin, soft, waxy, adherent, sulphine yellow (R) to naphthalene yellow (R) when fresh; margin indeterminate, pruinose, whitish to concolorous; spines concolorous with the hymenium, slender, elongated, 0.5–1 mm. long, solitary or slightly confluent, crowded, apices entire or denticulate; hyphae of the subiculum very compact, 2.5–3 $\mu$ in diameter, flexuous, septa few; hyphae of the subhymenium thinner walled, abundantly septate, clamp connections present; hyphae in axes of the spines adherent, very compact, 3–4 $\mu$ in diameter; basidia 13–16×4–5 $\mu$, clavate; spores 4–6×2.5–3 $\mu$, hyaline, oblong cylindrical and often containing two shining drops; cystidioles subulate, projecting 6–10 $\mu$ beyond the hymenium, 2–3 $\mu$ in diameter. After rejuvenation the spines may be densely bristly with hyphae or cystidioles (?) 2–3 $\mu$ in diameter.

Nine collections were made in Michigan of which no. 36 was determined by BOURDOT. It occurs on rotten hardwood logs, and is found more frequently on *Fraxinus nigra* than on other hardwoods.

When in good growing condition this fungus has a strong anise odor and exhibits a striking color reaction to alkali (**5**). Treating sections with potassium hydroxide produces a reddish violet color. Exposure of pieces of a specimen to the fumes of ammonia produces a violet color. BOURDOT and GALZIN (**5**) have distinguished *Odontia* (*Acia*) *uda* from *O.* (*Acia*) *fusco-atra* by this reaction.

Six monosporous cultures were made of *Odontia uda*. The mycelium produced by the single germinated spores was 2.5–4 μ in diameter, septate, and had clamp connections on the septa. Chlamydospores 8–15 μ in diameter were abundant in the cultures. The basidia were oblong-clavate, 15–30×3–4 μ in diameter. All six monosporous cultures formed clamp connections, produced basidia and basidiospores, and *Odontia uda* is therefore a homothallic species.

Small penicillate spines were produced on the upper surface of filter paper pads moistened with different nutrient solutions. The spines consisted of clusters of spreading, upright, slightly incrusted hyphae. The lower part of these spines was covered with the hymenium. The cultures produced a very strong, characteristic odor of benzaldehyde rather than the anise odor of fresh specimens. There was no color reaction when the mycelia of the cultures were exposed to the fumes of ammonia as was noted for material collected in the field.

*Odontia stenodon*

*Odontia stenodon* (Pers.) Bres.,–Atti. Accad. Rovereto III. **3**:96. 1897.

*Mycoacia stenodon* (Pers.) Donk,–Med. Ned. Myc. Ver. **18–20**: 151. 1931.

*Acia stenodon* (Pers.) B. & G.,–Hym. de France p. 415.

*Oxydontia stenodon* (Pers.) Miller,–Mycologia **25**:294. 1933.

Fructification effused, in patches 10–15×8–10 cm., adnate, waxy, cracking when dry, white to yellowish, pale salmon (R) when fresh, drying with yellow and reddish colors; margin fibrillose, radiating, narrow, white; spines often nodose, clustered or crowded, slender, connate at base, 1–3 mm. long; hyphae of subiculum very compact, agglutinated, 2.5–4 μ in diameter, moderately thick walled, the structure difficult to distinguish; hyphae in axes of spines compact, agglutinated, 3–4 μ in diameter, prolonged into sterile point or sometimes divided, apparently not incrusted, clamp connections present; basidia clavate, 15–24×3–5 μ, 2–4 sterigmata 2.5–3 μ long; no cystidia; cystidioles 2.5–3 μ in diameter on a level with the basidial layer or projecting 2–5 μ beyond; spores hyaline, oblong allantoid, 3.5–5.5×2.5 μ.

Three collections of this fungus were studied; one from New York, no. 35, which was determined by BOURDOT, and two from Michigan.

Both two- and four-spored basidia were found side by side in the hymenium on the same tooth.

A multisporous culture produced very little aerial mycelium but typical teeth were formed on the agar. Structurally the teeth were identical with those produced in nature. The hyphae of the submerged mycelium ranged from 1.5 to 4 $\mu$ in diameter, septate, with clamp connections. Incrusted, aerial hyphae were produced.

### *Odontia brinkmanni*

*Odontia brinkmanni* Bres.,–Ann. Myc. **1**:88. 1903.

*Grandinia brinkmanni* (Bres.) B. & G.,–Hym. de France no. 321. Bull. Soc. Myc. de France. 1914.

Fructification effused, forming patches 10–20 cm., thin, pruinose, adherent, white when fresh becoming yellowish on drying; margin subindeterminate; spines hemispherical, crowded, often conical and elongated; hyphae of the subiculum moderately loose, 1.5–4.5 $\mu$ in diameter, thin walled, often collapsing, clamp connections present; basidia urn-shaped, 4–8 spored with short sterigmata; spores hyaline, smooth, subelliptical, 4–6$\times$2–2.5 $\mu$; no cystidia; oxalate crystals abundant in the subiculum and spines.

This species was collected twice in Michigan, and one collection (no. 27) was determined by BOURDOT. It occurs on rotten hardwood logs.

In culture it produces a white to cream colored mycelium, at first chiefly submerged, later aerial and floccose. The mycelium of multisporous origin had clamp connections; however, it never produced basidia and basidiospores. Beadlike chains of thick walled chlamydospores were developed. Single chlamydospores were isolated and the mycelium produced was septate with clamp connections, white, and more floccose from the start than that produced from the basidiospores. However, no fructifications were produced.

*Odontia hydnoides* (figs. 2, 13)

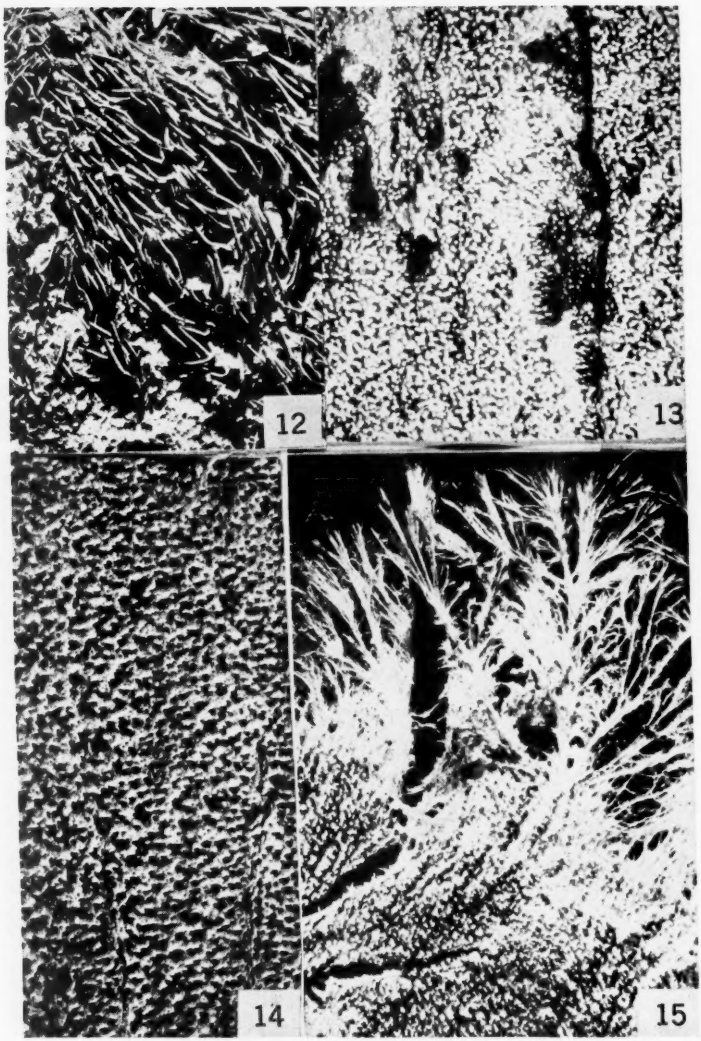
*Odontia hydnoides* (Cooke & Massee) v. Höhn.–K. Akad. Wiss. Wien. Math. Naturw. Kl. Sitzungsber. Vol. **118**:Abt. 1. p. 5–6. 1909.
Not *Odontia hydnoides* (Schw.) Peck,–N.Y. State Mus. Bull. 67. 39. 1903.

Fructification effused, in patches up to 40×15–25 cm., thin, adherent, waxy, crustaceous, pale gull grey (R) to pale smoke grey (R) with wax yellow (R) areas, finally at maturity light buff (R) or chamois (R); margin subdeterminate; spines concolorous varying from mere granules to teeth averaging from 0.5 to 1 mm. in length, subulate, fimbriate, with rigid bristles scattered or in fascicles as seen under the handlens, fragile, easily rubbed from the subiculum; hyphae of the subiculum compact, agglutinated, indistinct; cystidia abundant, strongly incrusted with oxalate, some with thick walls and narrow lumen, others with thinner walls and larger lumen, conical to fusiform, 53–100×10–21 μ, layered in the subiculum, partially imbedded in the spines or projecting at various angles and fascicled at the apices; hyphae in axes of the spines incrusted, septate, cylindrical, 8–12 μ in diameter, extending beyond the apices 20–40 μ, thinner walled than the cystidia; basidia 3.5–4 μ in diameter, length not determinable; spores 3–5×1.5–2 μ, oblong cylindrical, slightly flattened on one side, hyaline, usually with two guttulae. Incrustation soluble in hydrochloric acid.

The axillary incrusted hyphae have the power of regeneration. These hyphae, which are 10–12 μ in diameter, may from their apices give rise to hyphae 4–5 μ in diameter.

Specimens from England, Germany, France, and Holland were kindly loaned by DONK. A study of these, in comparison with 20 collections from Michigan and one from Louisiana, has convinced the writer that the American material is not specifically distinct from the European plants. The cystidia figured by VON HÖHNEL are very similar to those found in American collections.

In 1907 VON HÖHNEL and LITSCHAUER (**21**) described this fungus as a new species, *Peniophora crystallina*. In 1908 they (**22**) decided that this material was a delicate corticium-like variety of *Odontia conspersa* Bres., and reduced it to a variety, *O. conspersa* var. *crystal-*




FIGS. 12–15.—Fig. 12, *O. separans:* dried specimen collected by D. H. BELL, 1914. Fig. 13, *O. hydnoides* (no. 523) on rotten hardwood, Whitmore Lake, Mich. Gull grey (R) phase. Fig. 14, *O. bicolor* (no. 30) on coniferous wood, Nelson, N.Y. Determined by BOURDOT. Note darker tips to spines of dried specimen. Photograph by EDGERTON. Fig. 15, *O. fimbriata* (no. 427), Ann Arbor, Mich. Note fimbriate margin and exceedingly small, fimbriate spines. ×5.

*lina* (v. Höhn. & Litsch.). In 1909 VON HÖHNEL (**19**) called attention to three prevailing forms of this fungus, the odontia-like, the grandinia-like, and the peniophora-like forms. At the same time he reported on his study of COOKE and MASSEE'S original specimen of *Peniophora hydnoides* which is an *Odontia*, and therefore created the combination *O. hydnoides* (Cke. & Mass.) v. Höhn. That this fungus is decidedly variable can be seen from the treatment given it by different students. Thus VON HÖHNEL (**21**) and DONK (**16**) consider it a *Peniophora*, BRINKMAN (in VON HÖHNEL and LITSCHAUER **22**) a *Grandinia* BOURDOT & GALZIN (**5**), and CEJP (**13, 14**) an *Odontia*. The writer believes that it is an *Odontia* because of the well developed spines present in the mature fungus.

The color of this fungus is variable. Some specimens are gull grey (R) and others are light buff (R) to chamois (R). The gull grey specimens are young forms. The color change from gull grey to whitish or light buff was observed for two collections. Both colors have been noted on the same specimen in several instances. The average American collection shows some minor variation from the European material, in that the teeth are better developed, the cystidia have thicker walls and are stouter. There are gradations in the shape and size of the cystidia between collections of the American and of the European specimens.

The monosporous cultures of this fungus produced a thin, skinlike layer of mycelium over the surface of the agar. Around the margin of the petri dish and on the drier areas of the media in the test-tubes, thick, white, appressed, felty mycelium formed. The submerged hyphae were thin walled, 2–5 $\mu$ in diameter, and were slightly incrusted with small granules, becoming agglutinated into bundles but not forming rhizomorphs. Clamp connections were present but rare. Globose chlamydospores were abundant. They were 8–16 $\mu$ in diameter, thin walled at first, becoming thick walled in old cultures. They stained very dark brown with iodine solution or chloral hydrate with iodine.

The presence of clamp connections and the fruiting of monosporous cultures demonstrated the homothallic nature of this fungus.

It is slow to fruit in culture, although it is stimulated by a reduction in food supply. When KOTILA'S nutrient solution was replaced

by sterile distilled water in 10-day-old cultures on filter paper pads, fructification followed readily in 10–15 days. Check cultures were kept for 49 days with no signs of fruiting. When the nutrient solution was removed from these and replaced by sterile distilled water, basidia and basidiospores were produced in 11 days. The hymenium developed in culture had an irregular, crustlike formation and the typical, incrusted, fusoid cystidia characteristic of the field collections were not produced in culture in any case.

*Odontia bicolor* (fig. 14)

*Odontia bicolor* (A. & S.) Bres.,–Ann. Myc. **1**:87. 1903.

*H. balsameum* Peck,–N.Y. State Mus. Bull. 75. p. 15. 1904.

*H. serratum* Peck,–N.Y. State Mus. Rept. 50. p. 112–113. 1897.

Fructification broadly effused, variable, 8–60×5–20 cm., thin, adnate, subtomentose and pruinose to waxy and pruinose, white when fresh to milk-white (R) to cream color (R) when dry, cracking; margin determinate; spines conical to subulate, short, 0.5–1.5 mm. long, solitary or crowded, apices fimbriate, serrate on sides, on drying often forming fasciculate clusters especially when the teeth are long, apices often rufescent when dry; hyphae of the subiculum compact, interwoven, 1.5–2 $\mu$ in diameter, septate with clamp connections; hyphae in the axes of the spines thick walled, 5–8 $\mu$ in diameter, often agglutinated and slightly yellowish, extending beyond the apices of the spines 20–50 $\mu$, 3–5 $\mu$ in diameter and thinner walled; basidia clavate, 10–20×3–5 $\mu$, 2–4 sterigmata 4–5 $\mu$ long; spores oblong ovoid, apiculate, hyaline, smooth, 4–6 (8) ×3–4 $\mu$; cystidia of two types: one globose, spiny oxalate crystals 8–16 $\mu$ in diameter, incrusted on hyphae 1.5–2.5 $\mu$ in diameter, occurring on the sides and apices of the spines or in the subiculum near the substratum, incrustations soluble in hydrochloric acid; the other capitate cystidia, incrustations 7–9 $\mu$ in diameter on the sides and apices of the spines, cap part soluble in potassium hydroxide, alcohol, and chloral hydrate with iodine.

Specimens from New York, Michigan, Louisiana, Jamaica, and Holland were studied. The New York collection (no. 30) was determined by BOURDOT.

The types of *Hydnum balsameum* and *H. serratum* in the Herbari-

um of the New York State Museum, Albany, New York, show the same spiny crystals and capitate cystidia which are so characteristic of this species.

Not all attempts to get this fungus into culture were successful. Good spore deposits were secured in every case, but the spores would not germinate readily in distilled water or on agar at room temperature (20° C.). Twenty germinated single spores were isolated from material collected in Louisiana (no. 342) but only three (nos. 1–3) grew. The growth habit of no. 3 was markedly different from that of nos. 1 and 2, as the mycelial mat formed was from three to four times the size of the others. The mycelium from the no. 3 culture was more superficial, byssoid, and zonate whereas in the other two it was more floccose and azonate. These single spore cultures did not produce any clamp connections on the hyphae after a period of five months. The hyphae were moderately thick walled, 2–3.5 μ in diameter, uniform in size, with occasional irregular swellings, and were septate. No chlamydospores were formed. The mycelia of these were mated as follows: 1×3, 1×2, and 2×3. Clamp connections were formed in the case of 1×3. From these results it would seem that this species is heterothallic.

This fungus is exceedingly slow growing in culture and forms a mycelium mat of 3–5 cm. in diameter in a period of three months. Small, hyaline, rod-shaped segments 3–5×2–2.5 μ occurred in the single spore cultures, but when the aerial mycelium of the culture was examined under the 8 mm. lens, hyphae made up of many short segments were found, similar to what BREFELD illustrated as oidia. When water was added to the mount, these segmented hyphae disappeared and short, rodlike segments were abundant.

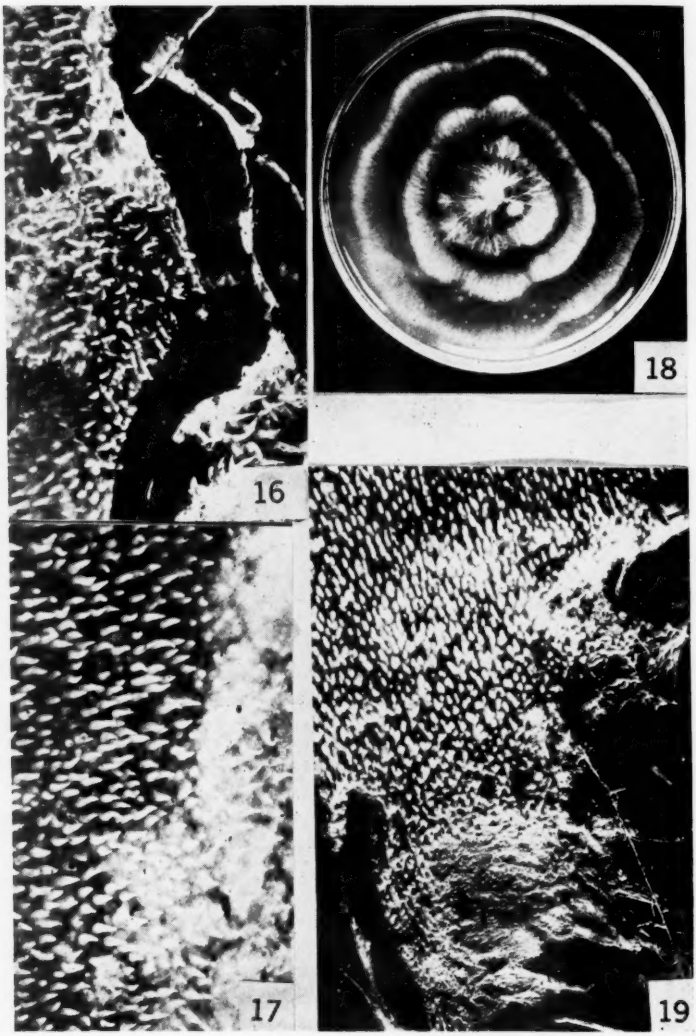
### *Odontia* **fragilissima** (figs. 18, 19)

*Odontia* **fragilissima** (B. & C.) comb. nov.

*Hydnum chrysocomum* Underw.,–Bull. Torr. Bot. Club **24**:82. 1897.

*Oxydontia fragilissima* (B. & C.) Miller,–Mycologia **25**:294. 1933.

Fructification effused, 2–6 to 60×15 cm., thin, fleshy-membranaceous when fresh, papery-membranaceous when dry, separable from the substratum, yellow to chrome orange when fresh; margin white,




FIGS. 16–19.—Fig. 16, *O. himantia* showing rhizomorph. Fig. 17, *O. himantia* (no. 545) showing acute and obtuse spines and cottony subiculum. Ann Arbor. Fig. 18, petri dish culture of *O. fragilissima* grown at 25° C. Note concentric zonations. Fig. 19, *O. fragilissima* (no. 429), Ann Arbor, Mich. Note spines and rhizomorphs. ×5.

byssoid, fibrous, usually with orange (R) to chrome orange (R) rhizomorphs; spines subulate, for the most part concolorous with the hymenium, apex usually lighter colored, 1–3 mm. long, some slightly flat and connate at the base, distant to crowded, much slimmer when dry than when fresh; hyphae of the subiculum 6–9 $\mu$ in diameter, incrusted with fine, orange colored granules, thick walled, compact, clamp connections present but rare; basidia 14–16×4–5 $\mu$ in diameter, 4-spored, very compact, arranged on the sides and between the teeth; spores oblong, hyaline, 3–5×2–2.5 $\mu$; cystidia, when present, finely incrusted, 3–6 $\mu$ in diameter, moderately thick walled and projecting 10–20 $\mu$; lumps of oxalate present in the axes of the spines; hyphae in axes of the spines agglutinated forming a compact bundle, some with very fine incrustations.

The following collections were studied: the co-type of UNDERWOOD'S species *Hydnum chrysocomum* in the Herbarium of the New York State Museum, also one other collection from New York, three collections from Michigan, three from Louisiana, one from Ohio, and one from North Carolina.

This plant can readily be recognized by its bright chrome-orange color, colored rhizomorphs, and the fragile, papery-membranaceous consistency of the dried specimens.

LLOYD (**29**) emphatically states that this species is *Sistotrema crocea* Schw., although he was unable to find any specimen of it in SCHWEINITZ' herbarium. It is difficult to see how LLOYD could mistake this well marked plant which is characterized by a thin, fragile, separable subiculum and colored rhizomorphs for a plant which was described as firm, tuberculose, rimose, and adnate.

BANKER (**1**) used the name *Hericium crocea* (Schw.) for plants which are parasitic on apple trees in North America and in Europe. The apple tree parasite is also known as *Hydnum schiedermayeri* Heuf., *Acia setosa* (Pers.) B. & G., and *Mycoacia setosa* (Pers.) Donk. DONK (**16**), however, believes that SCHWEINITZ' description does not refer to the apple tree parasite and that BANKER'S use of SCHWEINITZ' name for this fungus is unfortunate.

MILLER (**33**) placed UNDERWOOD'S name *Hydnum chrysocomum* in the synonomy of *Oxydontia fragilissima* but the writer believes the

plant belongs to the genus *Odontia* and has made the combination *Odontia fragilissima*.

A Louisiana collection (no. 332 from Pride, La.) consisted of a yellow colored specimen growing intermingled with an orange colored specimen on the same log and could not be distinguished from it other than by its color, as the microscopic details, presence of rhizomorphs, and consistency of the fruit bodies were identical. This yellow phase has also been noted by J. N. COUCH. With a specimen from Chapel Hill, North Carolina, he sent the following note: "This specimen varies from yellow to red, usually with orange rhizomorphs." In a later collection (no. 429) the chrome-orange fruit body was cut off from the wood and the bare stick placed in a moist chamber. In a few days it was covered with a whitish mycelium which soon became chrome-orange. After three to four weeks the color changed to capucine yellow (R), hence the yellow and orange phases were definitely connected.

A tissue culture from one of the Michigan collections was secured from rhizomorphs. It first produced a white, fibrous, aerial mycelium with hyphae 6–8 $\mu$ in diameter which had clamp connections. The mycelium was at first hyaline, then chrome-orange. The color is due in part to the fine granular incrustations which sheathe the hyphae, and in part to color in the protoplasm. The color is partly bleached out of the mycelium by potassium hydroxide, Amann's mounting medium, or chloral hydrate with iodine. The granules are somewhat loosened by these reagents; however, hydrochloric acid does not appear to attack them. Chloral hydrate with iodine stains the hyphae dark brown. Cultures grown in light on agar in flasks at room temperature produced concentric zonations around the point of inoculation, while cultures grown in darkness produced a fibrous aerial mycelium with abundant rhizomorphs. Petri dish cultures kept in the dark at 20°, 25°, and 30° C. produced concentric zonations similar to those formed in light (fig. 18). Here the zonation was broader and consisted of rings of aerial mycelium alternating with the submerged mycelium. All attempts to stimulate this fungus to fruit, such as reducing the food supply, changing the carbohydrate-nitrogen ratio, growing it at different constant temperatures and alternating high and low temperatures, failed.

Sixteen monosporous cultures were secured from no. 429, a Michigan collection. The primary mycelium consisted mostly of submerged hyphae of irregular size with swellings and contortions. About three weeks after isolation, the mycelium produced clamp connections, thus indicating a homothallic condition for this fungus. The secondary mycelium was more regular in diameter than the primary mycelium, and did not have the anastomosing network of branches found in the primary mycelium. The mycelium of the single spore cultures behaved like the mycelial cultures from the rhizomorphs in that after six months neither basidia nor basidiospores were formed.

### *Odontia* **separans** (fig. 12)

*Odontia* **separans** (Peck) comb. nov.

*Hydnum separans* Peck,–N.Y. State Mus. Rept. 50. p. 112. 1897.
*Oxydontia macrodon* (Fr.) Miller,–Mycologia **25**:294. 1933.

PECK described this species as follows:

> Resupinate, white; subiculum membranous, at first pure white, becoming yellowish or cream color with age; aculei subulate, glabrous, crowded, 2–3 lines long, fragile, easily separating from the subiculum and leaving in it alveolar impressions; spores globose, colorless, 0.00016 inch broad.
>
> Much decayed wood of deciduous trees. Adirondack Mountains. July.
>
> After the teeth have been separated from the subiculum, it resembles somewhat a shallow-pored species of *Poria*. By this character, the thinner subiculum and the smaller spores the species may be separated from *H. mucidum*, to which it is allied.

PECK reported the spores as globose, colorless, 0.00016 inch broad (4.06 $\mu$). Examination of the type specimen in the Herbarium of the New York State Museum indicated that the spores are broadly ellipsoid, amyloid, and that two types of gloeocystidia are present. The alveolar impressions which PECK noted are common also on other species of *Odontia* when the teeth break away from the subiculum.

MILLER (**33**) has placed PECK's name *Hydnum separans* in the synonomy of *Oxydontia macrodon* (Fr.) Miller (*Hydnum macrodon* Fr.). This treatment seems unwarranted because the Friesian de-

scription indicates a plant with almost no subiculum, whereas PECK's plant has a well developed subiculum. For this reason PECK's name is retained here.

Fructification effused, at first small, orbicular patches, 2 cm. in diameter, later up to 7×4 cm., soft, membranaceous, separable, white when fresh, colonial buff (MP) to amber yellow (MP) when dry; margin indeterminate; spines up to 6 mm. long, subulate, crowded, fragile, easily separated from the subiculum leaving small pits; subiculum moderately dense, hyphae of two kinds, one moderately thick walled, 2.5–4 μ in diameter, often collapsing, septate and with clamp connections, the other very thick walled, 2 μ in diameter, walls so thick and lumen so narrow that they sometimes appear like capillary hyphae; basidia cylindrical to clavate, 16–24×7–10 μ; sterigmata 3–5 μ long; spores smooth, hyaline, broadly ellipsoid, amyloid, 3.5–5-×3–4 μ; gloeocystidia of two types: one fusoid with acute to acuminate apices, the other in the axes of the spines thin walled, flexuous, 125–150 μ long, 7–10 μ wide, staining dark brown by chloral hydrate with iodine and reddish by potassium carbonate with eosin; spores turning blue under the action of iodine.

Two collections besides the type were studied, one from the vicinity of Ann Arbor, Mich., and one from Hemlock Ravine (? New Richmond, Mich.), collected by D. H. BELL, 1914, and in the Herbarium of the University of Michigan (fig. 12). The above description is based on these three collections.

Eight monosporous cultures and one polysporous culture of this species were made. The mycelia of the monosporous cultures did not have clamp connections. The mycelium of the polysporous culture showed clamp connections. Therefore, the fungus is apparently heterothallic. It has not fruited in the two years it has been in culture. The mycelia of the single spore cultures have hyphae of two sizes, one 1.5 μ in diameter, thick walled, the other 2.5–3 μ in diameter, thick walled, septate. Thick walled, intercalary chlamydospores are produced on hyphae of monosporous cultures. These vary from ellipsoid, 4–6×2–5 μ, to globose up to 8 μ in diameter. Some granular oxalate incrustations were produced on the hyphae. No gloeocystidia were produced in culture.

*Odontia himantia* (figs. 16, 17)

*Odontia himantia* (Schw.) Bres.,–Ann. Myc. **1**:84. 1903.

*Hydnum subfuscum* Peck,–N.Y. State Mus. Rept. 40. p. 55. 1885.

Fructification effused, size irregular depending upon the substratum, cottony tomentose, thin, at first white, then antique gold (MP) to fuscous when old, separable when fresh, sometimes slightly adherent; margin byssoid, determinate, usually with conspicuous rhizomorphs; spines up to 4–5 mm. long, scattered or gregarious, slender, obtuse or conical (often drying conical), entire, white when fresh to fuscous; hyphae of subiculum 2.5–3.5 $\mu$ in diameter, smooth, or minutely roughened, septa and clamp connections present; axillary hyphae mostly thin walled, 2.5–4 $\mu$ in diameter, agglutinate; basidia clavate, 2–4 spored, 18–38×5–8 $\mu$ in diameter; sterigmata subulate, straight, 6–7 $\mu$ long; spores cylindrical, often apiculate, hyaline, smooth, size variable depending upon maturity, 8–12 (14)×3–5 $\mu$; no cystidia.

Common on old, rotten frondose or coniferous wood, especially oak. Mycelium of this species appears in the soil and on débris in mid-summer. Fruiting specimens can be found as early as the middle of August, although the best development is later in the fall.

An examination of the type of *Hydnum subfuscum* Peck confirmed KAUFFMAN'S (**25**) belief that PECK'S species is the same as *Odontia himantia*. PECK commented on the similarity of *H. subfuscum* to *H. himantia* but believed that the acuteness of the spines in *H. subfuscum* denoted a specific difference. The occurrence of obtuse and acute spines on the same plant has been noted and moreover the obtuse spines often become acute on drying.

BOURDOT and GALZIN (**5**) have placed this species in the genus *Clavaria* under the section Ceratella. However, the hymenium occurs between and continuous with the spines and thus it properly belongs in the Hydnaceae.

*Odontia fimbriata* (fig. 15)

*Odontia fimbriata* Fr.,–Genera Hymenomycetum 13. 1836.

*Mycoleptodon fimbriatum* (Fr.) B. & G.,–Bull. Soc. Myc. France **30**:276. 1914.

*Gloiodon fimbriatum* (Fr.) Donk.–Ned. Bot. Ver. **1**:79. 1930.

*Etheirodon fimbriatum* (Fr.) Banker,–Bull. Torr. Bot. Club **29**:441. 1902.

Fructification effused, size variable, 8–20×5–10 cm., or in small patches, thin, membranaceous when fresh to papery or slightly coriaceous when dry, separable; nude (MP) to onion-skin pink (MP); margin fimbriate and with conspicuous rhizomorphs; spines small, short, conical, crowded, apices appearing bristly under a handlens; hyphae of the subiculum of two kinds, one moderately thick walled, rarely septate, 2–4 μ in diameter, the other thin walled, 3–5 μ in diameter, septations frequent, clamp connections of the same diameter as the hyphae; basidia cylindrical, clavate, 13–18×2.5–3.5 μ; spores oblong-ovoid, smooth, hyaline, 3–4×2 μ; cystidia cylindrical, thick walled, septate, 6–19 μ in diameter, projecting beyond the spines 10–50 μ, heavily incrusted with oxalate, soluble in hydrochloric acid. (Potassium hydroxide aids in distinguishing these better than do other reagents.)

Widely distributed on rotten wood. Specimens from Michigan, Pennsylvania, New York, and Ohio were studied.

BOURDOT and GALZIN (**4**) transferred this species to the genus *Mycoleptodon* because they consider the plant coriaceous and because it has thick walled hyphae making up the subiculum and cystidia similar to those in *M. ochraceum* (Pers.) B. & G. It is not so coriaceous as *M. ochraceum*, however, nor has it ever been found to be effuso-reflexed or dimidiate as are other species included in *Mycoleptodon*, and therefore should be placed in *Odontia*. There are other species of *Odontia* with thick walled, flexuous hyphae and cystidia similar to those in *O. fimbriata*.

### *Odontia setigera*

*Odontia setigera* (Fr.) Miller,–Mycologia **26**:19. 1934.

*Kneiffia setigera* Fr.,–Genera Hymenomycetum 17. 1836.

*Kneiffia setigera* Fr. Bresadola,–Ann. Myc. **1**:103. 1903.

*Odontia vesiculosa* Povah non Burt.,–POVAH, Papers Mich. Acad. Sci., Arts, Letters **9**:262. 1929.

Fructification effused, in small patches 1–2 cm. on a side, coalescing to form areas 15–30×8–20 cm., separable when fresh and slightly so when dry, very much cracked when dry, whitish when

fresh, agate grey (MP) to ivory (MP) when dry, finally becoming buff (MP) in the herbarium; margin arachnoid, pubescent, subdeterminate to determinate; spines variable, hemispherical to obtuse or cylindrical, less than 0.5 mm. long; hyphae of the subiculum loosely interwoven next to the substratum, thin walled, 3–5 μ in diameter; basidia 16–24×4–6 μ; sterigmata 4–6 μ long; spores oblong-cylindrical, hyaline, 6–12×3–4 μ; cystidia imbedded in the axes of the spines and projecting 30–80 μ, 6–10 μ in diameter; irregular, incrusted with oxalate crystals; cystidia, under the handlens appearing as shining "setae," thin walled, septate, clamp connections on the septa revealed after the incrustation has been dissolved in hydrochloric acid.

On rotten wood, Michigan, Iowa, New York, Louisiana.

BURT gave several herbarium names to this fungus, one of which was *Odontia vesiculosa* based upon a collection by A. H. POVAH from Vermilion, Michigan. POVAH (**37**) published the name "*Odontia vesiculosa* Burt, ined.," and gave the host and spore measurements. An examination of his collection in the Herbarium of the University of Michigan has shown that it is *Odontia setigera*.

## Discussion

One of the outstanding results of this study is the discovery that a majority of the species are homothallic. BLAKESLEE (**2**) first reported the occurrence of heterothallism in fungi among the Phycomycetes. Later this phenomenon was found in the Ascomycetes and Basidiomycetes. In the latter, heterothallic forms have been demonstrated among the Uredinales, the Ustilaginales, and the Agaricales. Only a few species of the Basidiomycetes which have been investigated have been homothallic. GÄUMAN and DODGE (**18**), in discussing sexuality of the Basidiomycetes, state "true homothallism is rare" although "it has been experimentally demonstrated" in certain agarics.

*Odontia fusco-atra*, *O. stenodon*, *O. uda*, *O. arguta*, *O. hydnoides*, and *O. fragilissima* all proved to be homothallic. Strains from single basidiospores produced cultures which had clamp connections on the mycelium and these cultures formed basidia and basidiospores. *O. bicolor* and *O. separans* apparently are heterothallic species. Strains from single basidiospores remained sterile, and the mycelia,

when mated in certain combinations, produced clamp connections. A sufficient number of monosporous cultures were not obtained to justify conclusions covering sexuality.

As was previously mentioned, microconidia, macroconidia, chlamydospores, bulbils, and oidia have been reported by various investigators of these fungi. In cultures of the homothallic species, no bulbils, microconidia, macroconidia, nor oidia were observed. Chlamydospores were present in cultures of both homothallic and heterothallic species. *Odontia bicolor* produced oidia on the mycelium derived from a single spore. Examinations of European and American collections of *Hydnum erinaceus* Bull. and *H. coralloides* (Scop.) Fr. failed to show the macroconidia or microconidia reported by DE SEYNES or by PATOUILLARD. The grapelike clusters of spores which they interpreted as conidia were seen, but when potassium hydroxide was added to the mount, the spores separated and a bare hypha was revealed, around which the spores were clustered, and this could not be interpreted as a conidiophore. When iodine was added, these spores turned blue, as did the spores attached to the basidia. The walls of the basidiospores of these species are viscid, as shown by the pronounced adherence of the spores to each other and to the hymenium. It seems probable that the microconidia and macroconidia which DE SEYNES and PATOUILLARD described are in reality basidiospores adhering in clusters.

The artificial environment of these fungi in pure culture on different types of media had a marked effect upon the fructification produced. Only two species produced fructifications similar to those formed in nature; namely, *Odontia arguta* and *O. stenodon*. These produced typical spines on nutrient agar, on filter paper pads in nutrient solution, and on woods wet with different nutrient solutions. All of the other species which fruited in culture produced granular crustlike fructifications with no regularity. Thus one of the diagnostic characters of the genus *Odontia*, the presence of a hymenium upon spines, may be decidedly modified or eliminated in artificial culture.

The external appearance of the fructification of *Odontia arguta* in culture was similar to that in nature. However, no characteristic cystidia were developed in culture. The formation of cystidia could

not be induced in cultures of different ages by varying the conditions of heat, light, moisture, and substratum. This lack of cystidia was noted in all cultures, with the exception perhaps of *O. fusco-atra*. This fungus produced scattered, individual, incrusted hyphae (or cystidia?) which projected beyond the hymenial layer. There was no tendency to form a spine around these hyphae, as occurs in nature.

The mycelium of *Odontia uda* in culture did not turn violet color when exposed to the fumes of ammonia as did field collections, and the anise odor of fresh field collections could not be detected in the cultures.

The classification of the resupinate species of the Hydnaceae has undergone various changes with the emphasis that different investigators have placed on the diagnostic characters such as consistency of the fructification, presence or absence of cystidia or other sterile organs, character of the spines, and color of the spores.

The consistency characters of the fructification such as fleshy, waxy, membranaceous, crustaceous, and coriaceous are rather difficult to interpret in these fungi. In fact several students, in describing the same species, have ascribed different consistencies to it. The use of consistency characters for delimiting genera seems to be of doubtful value.

The presence of cystidia is used extensively as a diagnostic character in the classification of fungi. In the Agaricaceae and the Polyporaceae, however, the occurrence of cystidia is not considered of sufficient value to separate genera. BURT (**8**), in his work with the Thelephoraceae, used the presence of cystidia to separate *Peniophora* from *Corticium*, but did not consider the presence of gloeocystidia a generic character. An example of the artificial classification which has resulted from the use of the occurrence of cystidia to separate genera is afforded by the genera *Mycoleptodon* and *Pleurodon*. BOURDOT and GALZIN (**4, 5**) used the presence of cystidia as one of the characters in *Mycoleptodon* to separate it from *Pleurodon*. In 1932 BOURDOT (**3**) directed attention to the fact that cystidia occur in one species of *Pleurodon* and are absent in several species of *Mycoleptodon*. He therefore united these two genera. Also the distribution of cystidia in a specimen is often variable, occurring in some portions

and not in others. The use of such a variable character to delimit genera results in an artificial classification and tends to separate closely related species.

The aculei vary from mere hemispherical granules to large subulate spines. They are scattered or gregarious on the subiculum, and often slightly confluent at their bases, especially where the spines are crowded or where the fructification grew on the side of a log. The apices of the spines may be obtuse to acute, or fimbriate or dentate. Drying of specimens causes a distortion of the spines and often a fasciculate clumping. The tendency has been to use the clumping of the spines, their coalescence, and the types of the apices as generic characters. For example, *Odontia* has been characterized by fimbriate or penicillate apices to the spines and *Acia* by entire tips to the spines. However, rejuvenation of the hyphae in the spine following a period unfavorable for growth will cause a fimbriate appearance to an otherwise entire spine. The size and character of the spines vary with the age of the specimen. The fasciculate clumping or molariform type of tooth results from drying in most instances.

The echinulate spore has also been used to delimit genera. In this group, however, there are only a few species with hyaline echinulate spores, and in most instances the markings are not readily visible. The use of this character for generic distinction in the Hydnaceae would result in the establishment of new genera with the same morphological characters as the existing genera, with the exception of the spore marking.

The amyloid condition of the spore has also been used as a generic separation. It also separates otherwise closely related species.

The classification of BOURDOT and GALZIN (**5**) was chosen as the best treatment to follow when these studies were started in 1929. However, the results obtained did not justify following this classification completely. They recognize four genera of the "Hydnés" as being always resupinate: *Radulum*, *Grandinia*, *Acia*, and *Odontia*. As has been indicated, these genera are differentiated by characteristics that separate closely related species. DONK (**16**) also considers that these genera are artificial and should be combined. As a result of these studies, the following emendation of the genus *Odontia* is proposed:

**Odontia** Fr., emended.

Synonyms: *Mycoacia* Donk; *Acia* Karst.; *Grandinia* Fr.; *Gausapia* Fr. pro parte; *Radulum* Fr. pro parte; *Kneiffia* Fr. pro parte; *Oxydontia* Miller.

Receptacle resupinate; consistency varied, membranaceous, crustaceous, waxy, arachnoid, soft or floccose; covered with granules, persistent, hemispherical, or with spines, conical or irregular, apices entire or penicillate; cystidia, gloeocystidia, and cystidioles present or absent, incrusted or naked; basidia 2–4–6–8 spored; spores hyaline, smooth or minutely echinulate, amyloid or non-amyloid. The type species is *Odontia fimbriata* Fr.

The type of the genus *Radulum*, *Radulum aterrinum* Fr., is an Ascomycete belonging to the genus *Eutypa* according to VON HÖHNEL (**20**). Many of the species since placed in the genus *Radulum* are typical odontias.

The type of the genus *Kneiffia*, *Kneiffia setigera* Fr., has been placed in the Thelephoraceae and in the Hydnaceae by different authorities, but is, in its best stage of development, a true odontia according to MILLER (**34**), who has transferred it to this genus.

The genus *Grandinia* Fr. is usually characterized by the hemispherical granules and by the absence of cystidia. As heretofore indicated, the shape and size of the spines and the absence of cystidia are characters too variable to be used for generic delimitation. Those who recognize the genus *Grandinia* admit that its separation from *Odontia* is artificial. The name *Grandinia* is antedated by *Gausapia* Fr., which, from its use in the Genera Hymenomycetum, should be the valid name if the segregation were maintained.

The genus *Acia* Karst. is usually separated from *Odontia* by the waxy consistency of the fructification and by the entire apices to the spines. Such a separation is artificial. Many of the species recognized in *Odontia* are described as waxy. The entire tip to the spine is very variable, even in the same fructification.

The name *Acia* is also not valid, as it has been used previously for a genus of the Rosaceae. DONK (**16**) proposed the name *Mycoacia* as a substitute for the invalid name *Acia*. Recently MILLER (**32**) proposed a new genus *Oxydontia* with the type *O. setosa* (Pers.) Miller.

The generic description, together with the species included in the genus, indicates that instead of a new genus a new name has been proposed for the non-valid name *Acia*.

## Summary

1. In studying species of *Odontia*, the use of reagents such as chloral hydrate with iodine and potassium carbonate with eosin has aided in distinguishing structures like cystidia, gloeocystidia, and subhymenial hyphae. The differential action of these reagents on spores, gloeocystidia, cystidia, and other hymenial elements offers supplementary characters for the identification of species. Likewise a knowledge of the corrosive action of these reagents on these structures is important. It has been shown that variations in descriptions of different investigators can in some cases be traced to the difference in the reagents used in the study of specimens.

2. The necessity for revising the generic concept is shown by this study, and an emended description of the genus *Odontia* Fr. is given. This genus is emended so as to include the species formerly included in the genera *Mycoacia* (*Acia*, *Oxydontia*), *Grandinia*, *Radulum*, and *Kneiffia*.

3. Two new combinations are made, *Hydnum fragilissimum* B. & C. and *H. separans* Peck being transferred to the genus *Odontia*.

4. A study of PECK'S types revealed that *Hydnum carbonarium* is the same as *Odontia fusco-atra; Hydnum serratum* and *H. balsameum* are the same as *O. bicolor*.

5. The following species were grown in pure culture for the first time: *O. fusco-atra*, *O. uda*, *O. bicolor*, *O. arguta*, *O. separans*, *O. fragilissima*, *O. hydnoides*, *O. stenodon*, and *O. brinkmanni*.

6. The following species produced basidia and basidiospores in culture: *O. fusco-atra*, *O. uda*, *O. arguta*, *O. hydnoides*, and *O. stenodon*.

7. Only two species, *O. arguta* and *O. stenodon*, produced fructifications similar to those produced in nature. Cystidia, an important diagnostic character, were not formed in culture.

8. The following seven species proved to be homothallic, mycelium from single spores developing clamp connections and producing

basidiospores: *O. fusco-atra*, *O. uda*, *O. arguta*, *O. fragilissima*, *O. hydnoides*, and *O. stenodon*.

9. *O. bicolor* and *O. separans* proved to be heterothallic, the mycelium from monosporous cultures lacking clamp connections which were formed only when two compatible strains were mated.

The writer expresses his appreciation to those who aided in these studies: to the late C. H. KAUFFMAN under whose guidance the problem was started; to E. B. MAINS under whose direction the studies were completed; to H. D. HOUSE for the privilege of studying PECK'S types; to ABBÉ H. BOURDOT and M. A. DONK for the numerous identifications and the loan of specimens; and to H. H. BARTLETT for editorial suggestions.

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# EFFECTS OF ENVIRONMENT UPON THE ROOT HABITS OF CERTAIN DECIDUOUS FOREST TREES[1]

HAROLD H. BISWELL

(WITH FOURTEEN FIGURES)

## Introduction

An experimental study of the development of roots and shoots of eight species of deciduous forest-tree seedlings was made near Fayette, Missouri, during 1933. Their growth in clay, alluvial, and loess soils was ascertained as well as the effects of shading and of deficient aeration. Root development of numerous saplings, 3 to 16 years of age, was also studied in different types of soil that occurred in relatively close proximity.

A study of the initial root habits of trees and their responses to changes in the external environment affords valuable criteria for judging the probability of the survival of a species when seeded on a particular soil type. It also leads to an understanding of natural reproduction as well as to an explanation of the course of succession. Since the area is part of a deciduous forest bordered by prairie, the findings are of particular interest in interpreting the relationship between forest and grassland.

Relatively little work has been done in America on the root habits of deciduous forest trees. CLEMENTS, WEAVER, and HANSON (**6**), working with seedlings of five deciduous species, found that the roots were greatly affected by different degrees of competition with grasses. AALTONEN (**1**) reached the conclusion that "the space arrangement of those parts of the trees which are above the soil is mainly decided by their roots and the competition existing between them for the water and food in the ground." BÜSGEN and MÜNCH (**5**), ROGERS and VYVYAN (**19**), and ADAMS (**2**) reached similar conclusions. Recently PARTRIDGE and VEATCH (**17**), SWEET (**22**), and YOCUM (**29**) in America, ROGERS and VYVYAN in England (**19**),

[1] Contribution from the Department of Botany, University of Nebraska, no. 84.

KVARAZKHELIA (**14**) in Russia, working with fruit trees, and NUTMAN (**16**) in Africa, working with coffee, have found that the roots are greatly influenced by soil conditions. GURSKY (**9**) has shown that even related species of mature trees may differ in their root habits, not only as to penetration into the soil but also as to root type. WEAVER and KRAMER (**27**) state: "Although the root habits of a tree are governed, first of all, by the hereditary growth characters of the species, they are often quite as much the product of environment."

HAASIS (**10**) showed that soil type greatly modified root form of western yellow pine seedlings. HEYWARD (**11**) found that soil texture influenced considerably the extent of laterals of longleaf pine. ANDERSON and CHEYNEY (**3**) concluded that in evergreens the soil texture affects the length chiefly of the tap root. STEVENS (**21**) ascertained that the rate of growth of roots of white pine was due to a combination of environmental factors.

That light and aeration play an important rôle in shoot and root development has been shown by TOUMEY (**23, 24**), HOLCH (**13**), HOFFMAN and SCHLUBATIS (**12**), and DEAN (**8**). Excellent resumés of the literature regarding root habit have been given by BÜSGEN and MÜNCH (**5**), LAITAKARI (**15**), and STEVENS (**21**).

## Material and methods

Seeds of the following trees were stratified out-of-doors in sand during the winter at a depth of 6 inches: *Gleditsia triacanthos* L., *Acer negundo* L., *Platanus occidentalis* L., *Juglans nigra* L., *Aesculus glabra* Willd., *Quercus maxima* (Marsh.) Ashe, *Hicoria ovata* (Mill.) Britton, and *Acer saccharum* Marsh. Early in March the seeds were planted in rows in cleared unshaded plots, one lot in upland clay, one in alluvial soil on a floodplain, and one in loess. When the seedlings began to appear one-half of each plot was shaded by lath frames. The frames were 2 feet high and covered on the top and south side with laths, each lath alternating with a space of similar width. For convenience, conditions under the frames will be designated as half shade. The seedlings were thinned so that they were 1.5–2 feet apart.

Growth in height was measured each two weeks. Number of

leaves, total leaf area, and dry weight of tops of representative samples were ascertained on July 1 and September 1. The form and extent of the root system of each species were determined in midsummer and also in early fall. Rates of transpiration under the two conditions were measured at one upland station from twelve individuals of each of five species grown in sealed containers. Environmental factors were measured regularly throughout the entire growing season.

Studies were made on the form and extent of the roots of 35 saplings. In nearly all cases isolated specimens were chosen, hence the roots were free from those of other trees. Several trees of each species were excavated.

## Ecological factors

### Precipitation

The mean annual precipitation is sufficient to permit the growth of an oak-hickory forest, which, as a result of clearing and agricultural practices, has now almost disappeared. Mean annual precipitation during the last 20 years was 37 inches. The greatest yearly rainfall was 50.9 inches, the least 26 inches. Normally the rain is well distributed throughout the growing season. During 1933, 3.6 inches fell in March, 2.6 in April, 7.8 in May, 1.3 in June, 0.8 in July, and 2.6 in August.

### Soil

The soils are diverse in nature although the most distant stations were only 18 miles apart. Their chief characteristics are shown in table I.

The clay soil on the gentle northwest slope became very hard as it dried with the advance of summer. The alluvial soil was that of the floodplain of Moniteau Creek. It is flooded for a few weeks annually and, as shown by the mottling of the deeper layers, is poorly aerated. The station with loess soil was near the crest of a ridge only a mile from the Missouri River. The land sloped gently northward.

Water content.—Soil samples were taken to a depth of 3 feet at all stations once each week from both the unshaded and shaded soil. The percentage of moisture was computed on the basis of oven-dry weight. Although the summer was dry, figure 1 shows that water

was always available for growth at all depths in the clay soil. In the unshaded loess soil the lowest recorded amount was 5.1 per cent in the surface 6 inches. With one exception, available water in the surface layer of alluvial soil always exceeded 10 per cent even under full insolation (table II). In fact, in the alluvial soil, water content was sometimes so high that it decreased aeration considerably.

TABLE I

TEXTURE, ACIDITY, HYGROSCOPIC COEFFICIENT, AND MOISTURE EQUIVALENT OF SOILS AT SEVERAL STATIONS

| SOIL, DEPTH IN INCHES | TEXTURE | | | pH | HYGROSCOPIC COEFFICIENT | MOISTURE EQUIVALENT |
|---|---|---|---|---|---|---|
| | CLAY | SILT | SAND | | | |
| Clay | | | | | | |
| 0–6 | 25.29 | 47.32 | 27.39 | 4.48 | 7.3 | 20.2 |
| 6–12 | 38.05 | 44.55 | 17.40 | 5.29 | 10.7 | 25.9 |
| 12–24 | 41.51 | 46.12 | 12.37 | 5.64 | 13.8 | 30.6 |
| 24–36 | 37.73 | 47.22 | 15.05 | 4.72 | 12.9 | 29.2 |
| Alluvial | | | | | | |
| 0–6 | 29.78 | 57.17 | 13.05 | 5.17 | 7.7 | 26.6 |
| 6–12 | 32.30 | 45.97 | 21.73 | 5.35 | 8.1 | 25.8 |
| 12–24 | 32.35 | 45.51 | 22.14 | 4.80 | 8.3 | 24.5 |
| 24–36 | 35.03 | 47.09 | 17.88 | 4.99 | 10.3 | 26.6 |
| Loess | | | | | | |
| 0–6 | 22.38 | 29.27 | 38.35 | 6.28 | 7.2 | 20.0 |
| 6–12 | 27.20 | 38.05 | 34.75 | 5.89 | 7.7 | 23.3 |
| 12–24 | 27.93 | 41.35 | 30.72 | 5.80 | 8.7 | 24.5 |
| 24–36 | 25.00 | 43.95 | 31.05 | 5.74 | 9.3 | 24.1 |

TEMPERATURES.—Temperatures of the soils were taken each week at various depths to 2 feet. They were obtained simultaneously in the open and shade at a station on sunny days. They were also measured hourly from 6 A.M. to 7 P.M. once each month. The results are summarized in table III. The loess soil was always slightly warmer than the clay or the alluvial soil, which usually had about the same temperature. The differences, however, were probably not great enough materially to affect the rate of growth in the different soil types.

## LIGHT

Light intensity was measured at noon on clear days by means of a Clement's photometer. The average light intensity under the lath

shelters was 33 per cent of full sunshine. The percentage of sunshine for the summer was very high. April and May had 57 and 62 per cent clear days, respectively; June, 89; and July, August, and September, 85, 65, and 73 per cent respectively.

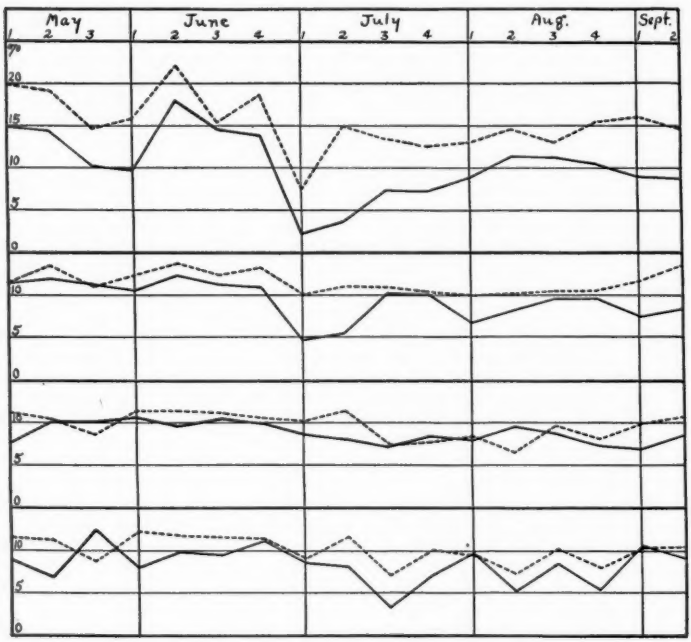


FIG. 1.—Water content in excess of hygroscopic coefficient in clay soil at depths of (upper lines) 0–6, 6–12, 12–24, and 24–36 inches. Solid line indicates full insolation, broken line half shade, and numbers the consecutive weeks of the months.

## HUMIDITY AND AIR TEMPERATURE

Humidity under the lath shelters was often 7 per cent higher than in the open, but differences of 3–4 per cent were more usual. Differences in humidity at the several stations were slight. Temperatures varied even less. They were usually 2° to 3° F. higher under full insolation, but maximum differences of 6° F. were found. Maximum differences in both humidity and temperature occurred between

noon and 4 P.M. Average daily maximum humidities during May and June were about 80 per cent and during July and August about

TABLE II

WATER CONTENT IN EXCESS OF HYGROSCOPIC COEFFICIENT AT VARIOUS DEPTHS AT THREE STATIONS THROUGHOUT THE SUMMER

| Station and amount | 0–6 Inches | | 6–12 Inches | | 12–24 Inches | | 24–36 Inches | |
|---|---|---|---|---|---|---|---|---|
| | Open | Shade | Open | Shade | Open | Shade | Open | Shade |
| Clay | | | | | | | | |
| Maximum | 17.8 | 22.1 | 12.3 | 13.7 | 10.9 | 11.8 | 12.4 | 12.0 |
| Average | 10.2 | 15.3 | 9.6 | 11.7 | 8.8 | 9.7 | 8.4 | 10.1 |
| Minimum | 2.1 | 7.6 | 5.5 | 10.0 | 7.1 | 6.4 | 3.5 | 7.0 |
| Alluvial | | | | | | | | |
| Maximum | 27.6 | 26.4 | 20.7 | 23.6 | 16.0 | 16.6 | 15.6 | 15.6 |
| Average | 16.0 | 17.5 | 12.7 | 16.2 | 13.2 | 13.9 | 12.1 | 12.5 |
| Minimum | 7.4 | 12.3 | 9.5 | 9.3 | 10.5 | 9.1 | 5.4 | 9.6 |
| Loess | | | | | | | | |
| Maximum | 15.7 | 18.9 | 16.5 | 16.6 | 15.4 | 16.0 | 16.3 | 16.4 |
| Average | 10.8 | 13.4 | 11.7 | 13.4 | 12.4 | 13.3 | 13.0 | 13.6 |
| Minimum | 5.1 | 7.7 | 7.6 | 9.5 | 10.0 | 9.0 | 10.3 | 10.3 |

TABLE III

AVERAGE AND EXTREME TEMPERATURES OF SOILS AT VARIOUS DEPTHS DURING THE SUMMER

| Soils and temperatures | 1 Inch | | 3 Inches | | 12 Inches | | 24 Inches | |
|---|---|---|---|---|---|---|---|---|
| | Open | Shade | Open | Shade | Open | Shade | Open | Shade |
| Clay | | | | | | | | |
| Maximum | 110.8 | 90.0 | 100.0 | 88.2 | 83.3 | 78.4 | 79.2 | 76.7 |
| Average | 98.1 | 83.3 | 90.5 | 79.4 | 77.4 | 73.3 | 73.5 | 70.4 |
| Minimum | 82.0 | 73.4 | 79.0 | 71.0 | 67.0 | 66.0 | 61.0 | 59.9 |
| Alluvial | | | | | | | | |
| Maximum | 111.3 | 89.6 | 101.2 | 87.8 | 83.2 | 80.5 | 82.0 | 78.8 |
| Average | 96.0 | 81.9 | 89.9 | 79.3 | 77.1 | 73.6 | 73.5 | 71.3 |
| Minimum | 77.0 | 68.0 | 76.2 | 67.6 | 66.0 | 64.4 | 61.0 | 60.0 |
| Loess | | | | | | | | |
| Maximum | 112.3 | 100.4 | 98.2 | 88.2 | 87.4 | 85.1 | 82.4 | 80.5 |
| Average | 99.4 | 84.2 | 90.6 | 79.8 | 78.1 | 74.2 | 75.1 | 72.1 |
| Minimum | 79.7 | 70.7 | 74.3 | 68.0 | 65.3 | 60.8 | 59.9 | 59.0 |

75 per cent. The corresponding average daily minimum humidities for the months just given were about 54 and 43 per cent.

The mean maximum daily temperature for the last two weeks in May was 79° F. This increased in June to 91.5° and in July to 92.9°. It averaged 87.7° during August. The mean minimum daily temperature for the last two weeks in May was 58°, and for the other months as follows: June 63.1°, July 65.4°, and August 62.2°.

### Wind and evaporation

An average of 500 readings of wind movement, taken alternately in the open and under the lath shelters, gave a velocity of 609 feet per minute in the open but only 143 (77 per cent less) under the shelter. Average daily evaporation losses from Livingston's standardized, white spherical atmometers placed under full insolation and in the shade were (in cubic centimeters): May 28.9 and 20.7, June 41.4 and 31.9, July 49.1 and 38.2, August 29.4 and 22.7, September 23.5 and 15.7. For the entire summer the average in the open was 34.5 cc. per day and in the shade 25.8 cc. Greatest differences occurred on sunny days when the wind velocity was high.

## Observations

The eight species previously listed, except *Hicoria ovata* and *Acer saccharum*, were grown in all three soil types. The *Hicoria* was grown in the clay and loess soils and *Acer* in the clay and alluvial soils. The soil was cultivated lightly. Ninety-one trees were excavated by the trench method during midsummer and the root habits of 93 additional seedlings were studied in the fall. Saplings excavated consisted of the eight species enumerated (except *Aesculus glabra*) and in addition *Quercus macrocarpa* Michx. and *Populus sargentii* Dode.

### *Gleditsia triacanthos*

Seedlings.—The seeds of honey locust germinated readily, but the seedlings grew slowly in early spring. Growth was rapid during the sunny humid days of summer. Table IV shows that early growth was greatest in the clay soil. Here the seedlings were 16 per cent taller than in the loess soil and 57 per cent taller than on the floodplain. Growth in sun and shade was the same in the clay but differences occurred in the other soils. A maximum growth of 1 inch per day was sometimes attained under full insolation during mid-

TABLE IV

HEIGHT IN INCHES OF SPECIES AT THREE STATIONS IN FULL INSOLATION AND IN PARTIAL SHADE

| SOIL AND SPECIES | JUNE 1 | JUNE 29 | JULY 28 | AUGUST 25 | SEPTEMBER 7 |
|---|---|---|---|---|---|
| Clay | | | | | |
| Honey locust | 4.4 (4.4)* | 7.5 (7.1) | 9.7 (8.9) | 11.4 (10.5) | 11.4 (10.8) |
| Boxelder | 4.2 (4.1) | 5.9 (5.9) | 11.7 (12.2) | 16.2 (18.1) | 16.4 (18.2) |
| Sycamore | 0.8 (0.9) | 2.0 (2.7) | 5.4 (8.0) | 11.0 (15.4) | 11.0 (15.4) |
| Black walnut | 7.7 (8.5) | 9.0 (12.8) | 9.2 (14.9) | 9.2 (14.9) | 9.2 (15.0) |
| Buckeye | 1.8 (2.6) | 1.8 (2.7) | 2.4 (2.6) | 2.9 (2.8) | 2.9 (2.8) |
| Red oak | 4.1 (4.5) | 6.6 (7.5) | 7.8 (11.3) | 8.5 (11.3) | 8.3 (11.6) |
| Shellbark hickory | 4.2 (2.8) | 4.6 (5.4) | 4.6 (5.1) | 4.3 (5.0) | 4.3 (5.0) |
| Hard maple | 2.0 (1.8) | 1.7 (2.8) | 2.4 (4.0) | 2.2 (4.2) | 2.2 (4.1) |
| Alluvial | | | | | |
| Honey locust | 2.8 (3.1) | 5.5 (4.6) | 14.3 (8.8) | 22.0 (11.6) | 22.6 (12.1) |
| Boxelder | 3.2 (4.1) | 3.5 (4.5) | 5.3 (5.9) | 6.8 (6.7) | 7.1 (6.9) |
| Sycamore | 0.8 (0.8) | 2.0 (1.6) | 6.7 (4.0) | 13.3 (10.0) | 13.8 (10.0) |
| Black walnut | 6.7 (6.7) | 10.4 (10.7) | 11.1 (12.6) | 11.6 (12.5) | 11.6 (12.5) |
| Buckeye | 1.6 (1.3) | 1.5 (1.2) | 1.2 (1.4) | 1.3 (1.5) | 1.3 (1.5) |
| Red oak | 3.5 (4.2) | 3.5 (4.5) | 9.0 (12.0) | 9.8 (12.9) | 9.9 (13.0) |
| Hard maple | 1.2 (1.3) | 1.2 (1.4) | 1.7 (1.8) | 2.1 (2.0) | 2.1 (2.0) |
| Loess | | | | | |
| Honey locust | 3.8 (3.1) | 9.6 (5.8) | 20.5 (11.0) | 30.5 (16.8) | 30.6 (16.8) |
| Boxelder | 4.8 (4.6) | 9.8 (7.5) | 14.0 (11.2) | 22.0 (18.8) | 23.3 (19.0) |
| Sycamore | 0.8 (0.6) | 3.5 (2.8) | 8.7 (9.6) | 16.0 (15.8) | 16.1 (15.8) |
| Black walnut | 7.2 (7.8) | 11.2 (14.0) | 12.2 (15.9) | 12.3 (16.8) | 12.3 (16.9) |
| Buckeye | 2.1 (1.7) | 2.0 (2.2) | 2.1 (2.4) | 2.7 (2.1) | 2.7 (2.1) |
| Red oak | 3.0 (2.9) | 3.9 (4.0) | 5.5 (4.8) | 5.3 (4.6) | 5.3 (4.6) |
| Shellbark hickory | 4.1 (3.2) | 4.2 (4.5) | 4.6 (5.3) | 4.4 (4.4) | 4.4 (4.4) |

* Height in partial shade given in parentheses.

TABLE V

DEVELOPMENT OF LEAVES AND TOPS AT MIDSUMMER AND AT END OF GROWING SEASON

| Soil and species | No. of leaves | | Area of leaves (sq. in.) | | Dry weight of tops (gm.) | |
|---|---|---|---|---|---|---|
| | Open | Shade | Open | Shade | Open | Shade |
| Clay | | | | | | |
| Honey locust | 9 (41)* | 9 (31) | 42.0 (70.5) | 30.4 (53.4) | 1.2 (3.6) | 0.6 (1.7) |
| Boxelder | 55 (49) | 47 (78) | 77.4 (264.4) | 71.0 (514.5) | 2.0 (11.8) | 1.7 (11.5) |
| Sycamore | 7 (46) | 13 (33) | 10.6 (233.3) | 30.0 (605.1) | 1.3 (6.7) | 1.8 (10.3) |
| Black walnut | 105 (110) | 130 (124) | 294.4 (448.5) | 442.2 (1115.9) | 8.1 (22.4) | 8.2 (39.9) |
| Buckeye | 14 (11) | 13 (16) | 6.2 (8.1) | 37.0 (30.8) | 0.2 (1.0) | 1.7 (1.8) |
| Red oak | 20 (18) | 20 (31) | 131.0 (202.2) | 179.8 (355.6) | 3.2 (8.7) | 4.2 (13.1) |
| Shellbark hickory | 9 (6) | 11 (12) | 35.4 (28.3) | 61.4 (34.1) | 1.2 (1.4) | 1.8 (1.6) |
| Hard maple | 10 (15) | 17 (17) | 8.2 (12.2) | 17.2 (47.8) | 0.2 (0.6) | 0.3 (2.0) |
| Alluvial | | | | | | |
| Honey locust | 14 (127) | 7 (33) | 36.4 (220.1) | 28.6 (57.6) | 1.3 (18.1) | 0.6 (3.3) |
| Boxelder | 30 (39) | 29 (53) | 16.2 (80.6) | 34.2 (118.1) | 0.7 (2.9) | 0.4 (2.9) |
| Sycamore | 7 (34) | 6 (11) | 22.4 (329.6) | 12.4 (261.2) | 0.5 (9.0) | 0.3 (4.5) |
| Black walnut | 121 (159) | 110 (116) | 380.8 (744.8) | 397.8 (716.1) | 10.1 (28.3) | 7.4 (16.1) |
| Buckeye | 14 (7) | 13 (7) | 26.4 (14.6) | 42.8 (25.7) | 1.6 (1.3) | 1.9 (1.3) |
| Red oak | 17 (23) | 16 (18) | 110.8 (229.6) | 178.0 (267.6) | 3.4 (9.7) | 4.3 (8.1) |
| Hard maple | 5 (11) | 5 (13) | 8.8 (23.1) | 2.6 (18.6) | 0.3 (0.9) | 0.1 (0.6) |
| Loess | | | | | | |
| Honey locust | 26 (143) | 11 (67) | 45.2 (388.9) | 19.1 (116.6) | 2.6 (25.9) | 1.0 (7.8) |
| Boxelder | 74 (158) | 55 (96) | 243.0 (974.5) | 140.0 (577.5) | 5.5 (27.5) | 2.6 (14.1) |
| Sycamore | 13 (28) | 12 (24) | 121.0 (418.4) | 78.0 (356.9) | 2.7 (10.6) | 1.4 (8.6) |
| Black walnut | 113 (150) | 121 (130) | 467.6 (1036.1) | 771.0 (1022.9) | 11.2 (38.1) | 14.3 (27.2) |
| Buckeye | 11 (3) | 13 (10) | 26.0 (5.8) | 39.8 (28.7) | 1.5 (1.3) | 1.3 (1.9) |
| Red oak | 20 (15) | 14 (13) | 77.2 (77.8) | 58.0 (52.0) | 2.4 (4.3) | 1.5 (2.5) |
| Shellbark hickory | 20 (15) | 12 (9) | 28.0 (31.4) | 32.1 (44.0) | 0.6 (2.6) | 0.7 (1.4) |

* Development of leaves and tops at end of growing season given in parentheses.

summer. The retarding effects of shade are clearly shown in table IV. Maximum growth, as measured by number of leaves, total leaf area, and dry weight of tops, occurred during July and August (table V).

The roots of seedlings were consistently more extensive under full insolation (fig. 2). By midsummer their depth of penetration averaged 25, 18, and 24 inches in the clay, alluvial, and loess soils respectively, as compared with 13, 14, and 9 inches in the shade. The

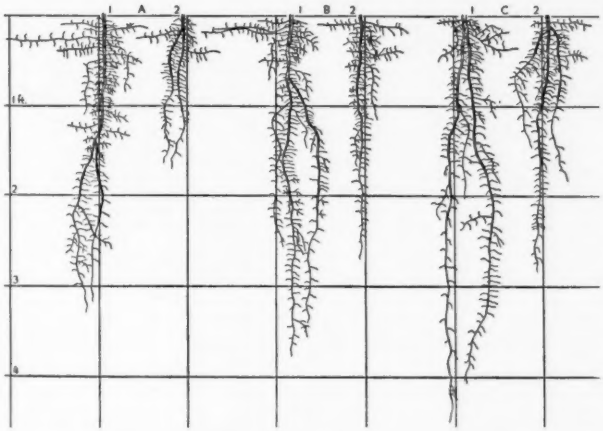


FIG. 2.—Root systems of seedling honey locusts excavated in fall from clay (*A*), alluvial (*B*), and loess (*C*) soils. In this and the following figures, (1) indicates trees growing in open and (2) those growing under lath shelter.

general form of these juvenile roots was the same in all cases. At the end of the season the greatest penetration was recorded in the loess and the least in the clay soil. The effects of shading were marked, especially in the clay soil where the retardation in root growth was 50 per cent.

SAPLINGS.—Four isolated saplings 4 to 6 years old and 4 to 7.5 feet tall were excavated, two from clay soil of upland and two from alluvial soil of low ground. The taproots had a diameter of 2 to 2.5 inches. They tapered gradually, pursued a somewhat tortuous course, and reached depths of only 20 to 24 inches in the moist soil of the lowland but 5 to 5.25 feet in the upland clay. An example of

their irregular course is illustrated by a taproot that turned at 3 feet in depth and ran obliquely upward for 25 inches, where it reached the 32-inch soil level; thereafter it pursued a vertically downward course.

Primary laterals were always abundant. From 6 to 15, exceeding 5 mm. in diameter, commonly arose from the first foot. They usually branched freely and often dichotomously. They were about twice as long in the upland as in the lowland soil, where many extended laterally 9 to 12 feet and a few over 17 feet. Usually 20 to

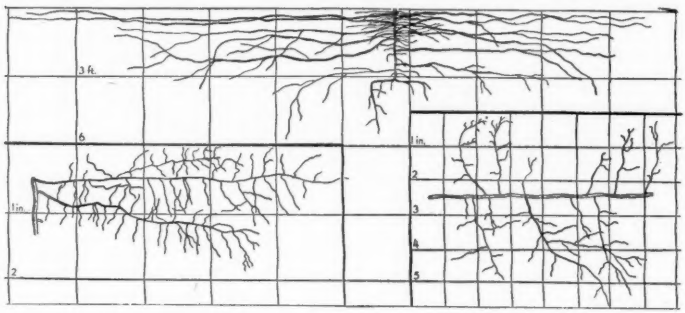


FIG. 3.—Six-year-old sapling honey locust excavated on upland (center) showing secondary laterals typical of those at 6-inch level (left), and laterals at depth of 3 feet (right).

30 smaller main laterals also arose from the first 6 inches of taproot and they occurred somewhat less abundantly to a depth of 2 feet. At greater depths they were sparse. Like the larger branches their course was mainly horizontal (fig. 3). Secondary laterals occurred at a rate of five to ten per inch of primary lateral. Tertiary branches were few and those of the fourth order rare. Even the smaller rootlets were long and of relatively large diameter. The brownish root hairs formed a thick covering on roots of the upland trees but were far less abundant on those in wet soil. The generalized, well developed root system enables this species to grow on upland as well as on lowland soil.

### *Acer negundo*

SEEDLINGS.—The seedlings of boxelder made a rapid early growth in the clay and loess soils but in the wet alluvial soil growth was re-

tarded (table IV). Leaf area in the loess plots was three times greater than that in the clay plots, but on the floodplain it was much less. Dry weight of tops showed similar relations (table V). Many of the lower leaves fell from the seedlings in the clay soil during the dry period in July and August. Consequently, in early fall the leaf area in the shade was about twice as great as in the open. Leaf area

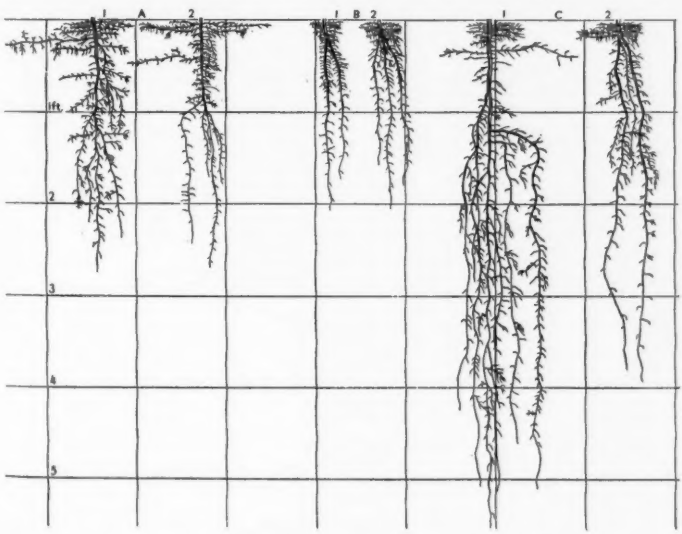


FIG. 4.—Root systems of 4-month-old boxelders grown in full sunshine and half shade in clay (*A*), alluvial (*B*), and loess (*C*) soils.

in the shade on the floodplain exceeded that in the open but dry weight of tops was the same under both conditions (table V). Number and area of leaves and dry weight of tops in the loess soil were almost twice as great in full insolation as in the shade.

The root systems showed similar development in midsummer, except in the wet alluvial soil where growth was much retarded. Their development by September is shown in figure 4.

SAPLINGS.—The root systems of two saplings about 4 inches in diameter, 13 and 15 years old and 13 and 15 feet high, were excavated near a creek where they were much shaded except on the north. Because of large lateral branches, the taproots decreased rapidly in

diameter to about 0.75 inch at 2 feet and 0.25 inch at 5 feet. The general course was not vertically downward but approximately that of a large spiral, the deviation from the perpendicular varying from 1.5 to about 3 feet. The maximum distance of the end of the taproot from a perpendicular line drawn from the soil surface was 27 inches. One taproot grew to a length of 9 feet, the other, after branching dichotomously at 7.5 feet, extended both branches to the 8.5-foot level where they ended in nearly saturated soil.

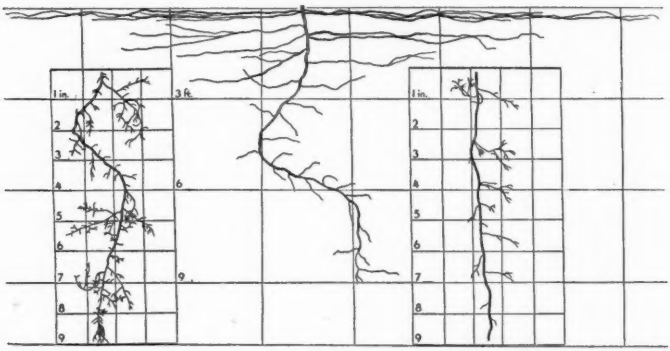


FIG. 5.—Root system of 15-year-old boxelder growing on floodplain (center) showing small portions representative of branching near soil surface (left), and at 3 feet (right). The same method of showing degree of branching of saplings is used in the following figures.

Three to six large laterals averaging about 0.75 inch in diameter arose from each taproot in the first foot of soil. They extended outward near the soil surface to a distance of 5–12 feet. Four or more other laterals, 0.75–1.5 inches thick, arose at greater depths but mostly in the surface 24 inches. They branched freely but did not spread so widely, usually only 2 to 8 feet. Their general course, like that of the shallower branches, was nearly horizontal. Frequently they divided into three or four parts of about equal size. Small primary laterals were few. Below 2 feet, all primary laterals were sparse, poorly developed, and little rebranched (fig. 5). Branches of the second and lower orders were most abundant within a few feet of the base of the tree.

The three isolated saplings examined in the clay soil of the up-

land were only 5 years old, 2 inches in average diameter, and 7 to 8 feet tall. Notwithstanding their immaturity and smaller tops,

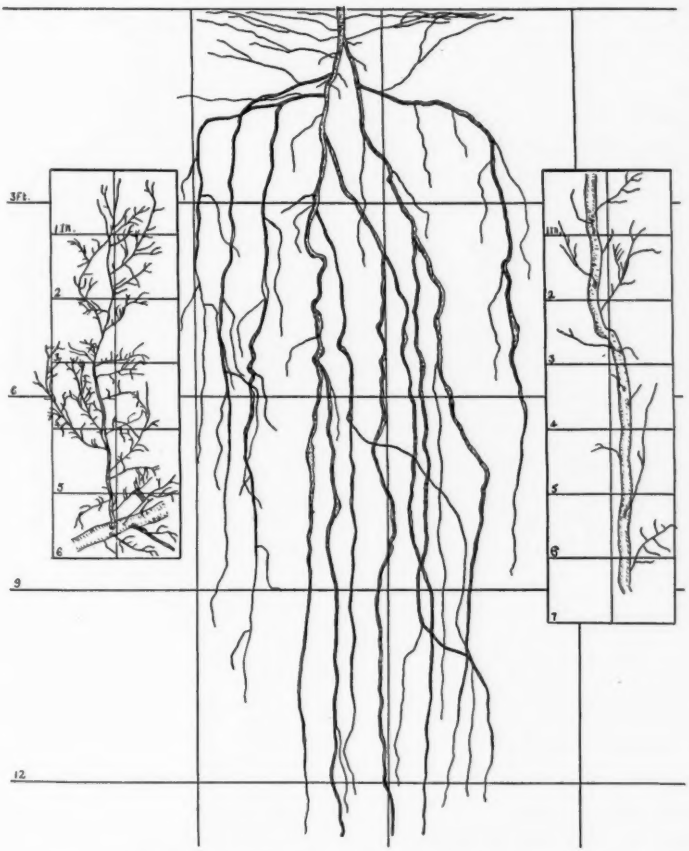


FIG. 6.—Root system of 5-year-old boxelder growing in upland clay soil. Note numerous surface roots and much distorted, deeply penetrating root system. Longest roots were traced only 12 to 13 feet but they penetrated more deeply.

their root systems greatly exceeded those of the floodplain trees. Figure 6, which is representative for the group, shows not only the greater depth of penetration, which was 12–13 feet, and the smaller

lateral spread of branches, which scarcely exceeded 4 feet, but also the vertical course and great depth to which many of the large laterals penetrated in this better aerated and drier soil. Even the smaller branches grew only a little outward and then turned downward. Close examination shows the tortuous course of the roots through the hard, rather dry clay. In cross section they often varied in shape from circular to an ellipse six times as long as wide. Below 7 feet, where the water content increased, the roots resumed a more regular shape and exhibited fewer curvatures. Finer roots were also noticeably more abundant here. On the upper portion of the taproot where the branches ran outward and upward in the surface foot, branching was especially profuse.

Grafting between roots of various sizes was found. It usually occurred where a small root crossed over or under one of larger size. Apparently the resistance between the growing roots was less than that between the roots and the soil. Where the roots of mature trees were partly exposed by soil erosion it could be seen that grafting was common.

### *Platanus occidentalis*

SEEDLINGS.—The sycamore seedlings grew slowly during the cool early spring and developed best during August (table IV). Leaf area and dry weight of tops were greater in the shade in clay soil; otherwise they were greater under full insolation. The roots were most extensive in the loess (3 feet), intermediate in the clay (2.2 feet), and least developed in the alluvial soil (1.9 feet). Shading caused a retardation in depth and also in the production of laterals.

SAPLINGS.—The root systems of four saplings 5 to 6 years old and 7.5 to 10 feet tall were studied. The largest had a crown diameter of 8 feet. They grew in a mellow loam soil underlain at about 2 feet with a tenacious clay. The strong taproot pursued a nearly vertically downward course, tapering gradually. One taproot, for example, was 4 inches thick at the soil surface, 3 inches at a depth of 1 foot, but only 0.2 inch at a depth of 5 feet. It branched dichotomously a little deeper and ended at about 7 feet (fig. 7). The roots were often distorted in penetrating the hard clay soil.

Usually five or more large primary laterals, 0.5–0.75 inch in

diameter, arose from the first 6 inches of the taproot. These spread widely, usually 5–9 feet, and branched freely in the surface 6 inches of soil. They were supplemented by primary laterals of smaller diameters. Major roots arising in the second 6-inch soil layer or deeper usually extended obliquely downward 1–2 feet, and then turning almost vertically downward penetrated to depths of 4–6 feet. A few, however, spread irregularly or horizontally outward. One at a depth of 2.5 feet, for example, ran nearly 5 feet laterally before turning downward. Upon this widely extending framework, absorbing roots occurred abundantly. A maximum rate of 32 branches per inch was found in the mellow surface soil. Between the depth of 6 and 18 inches a rate of branching of about 12 laterals per inch was determined. Except for branches of the first order, laterals were scarce below 25 inches. Here many of the primary laterals were free of branches for several inches.

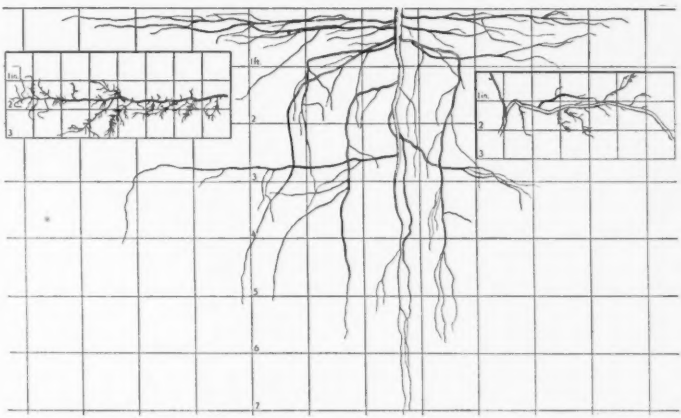

FIG. 7.—Typical root system of 6-year-old sycamore grown in loam soil

### *Juglans nigra*

SEEDLINGS.—Growth in height of the seedlings of black walnut was favored in every case by shading, the effects being greatest in the clay soil. The tallest seedlings, however, grew in the loess soil. The leaf area was more than doubled, and dry weight of tops was

increased 78 per cent under shade in the clay soil. Conversely, at the other stations the number of leaves, leaf area, and dry weight of tops were greatest under full insolation (table V).

The roots were very similar in all three soils early in July, although shading caused a retardation of 3–6 inches in length, the average depth being about 27 inches. A lateral spread of 12–24 inches was attained. By the end of the summer differences in the soil had caused marked changes in the form of the root system

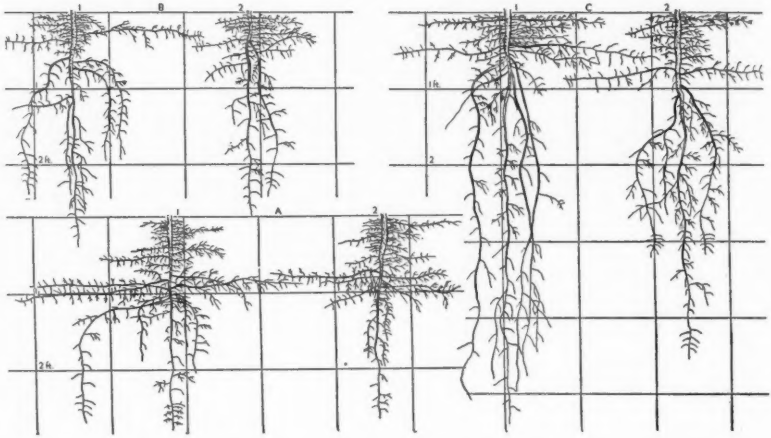

FIG. 8.—Roots of black walnut in clay (*A*), alluvial (*B*), and loess (*C*) soils in September of first growing season.

(fig. 8). Those in the clay spread widely, but in the loess, in addition to spreading widely, they penetrated far downward. In the alluvial soil they were somewhat intermediate in character.

SAPLINGS.—Three trees, 7 to 12 years old, growing in an upland clay loam underlain with a heavy clay subsoil, were selected for study. They varied from 2 to 6 inches in diameter, were 7–21 feet in height, and had a spread of tops of 5–10 feet. They were partially shaded by a second-growth forest. The plan of development of the taproot was similar in all in regard to rate of tapering, number of major laterals, and depth at which they divided into terminal branches (fig. 9). A taproot 2.25 inches in diameter decreased to 2 inches at 1 foot in depth and to 1 inch at 2 feet, where it branched into three parts, each

reaching a depth of 52 inches. The largest tree extended its taproot to 72 inches. The direct course of the roots was impeded by the hard soil, and the roots were crooked and often flattened.

The longer laterals occurred near the soil surface. On one tree, for example, six, each about 1 inch thick, arose from the first 4 inches of the taproot. Only one extended obliquely downward. It penetrated to a depth of 4 feet. Five extended directly outward along an almost horizontal course. In most cases the major laterals divided into two or three parts at a distance of 5 to 6 feet from their

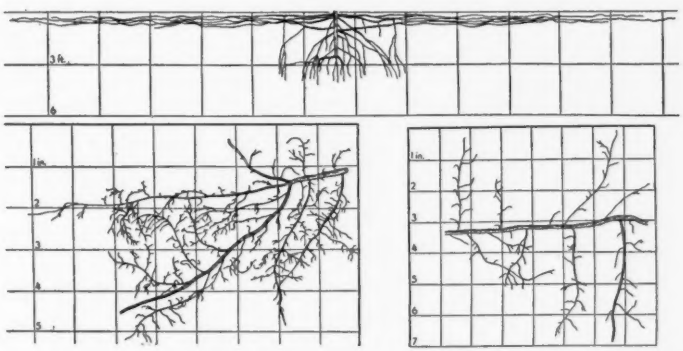

FIG. 9.—Root system of 7-year-old walnut in upland clay soil. Note wide spreading but shallow penetration of roots.

origin. These curved gracefully outward, tapered gradually, and spread widely. Some ended 16 feet from the base of the tree, one extended outward 18.8 feet, and the longest to 20 feet, which was three times the width of the entire crown of the sapling. On a tree 21 feet tall and 10 inches in diameter one of these horizontal laterals was traced outward 51 feet, where it was still 0.5 inch thick. All of these shallow roots were abundantly furnished with secondary laterals which averaged about 14 per foot but sometimes reached a maximum of 40. In many cases, however, only a few secondary branches were found on the initial 3–4 feet of main laterals.

A few vertically penetrating branches, often designated as sinkers, were found on some of these horizontal roots. They branched freely and penetrated into the third and fourth foot of soil. A few of the

deeper and smaller laterals extended upward and outward, some to within 3–4 inches of the soil surface, at 4–5 feet from the base of the tree. But most of these rather numerous laterals ran obliquely outward and downward throughout a course of 3–5 feet. None extended deeper than the taproot, or about 52 inches, and their lateral spread seldom exceeded 4 feet. Sublaterals were less abundant on these deeper branches, and were often threadlike and poorly rebranched.

*Aesculus glabra*

SEEDLINGS.—Most of the growth of the shoots of buckeye occurred in May. After the exhaustion of the food supply from the seed, development was greatly retarded. By fall they had added but little to the growth attained in June. Growth was slightly greater under full insolation in the clay and loess soils but less in the alluvial soil (table IV). Defoliation began to occur in July and by the middle of August some seedlings were completely leafless. Dry weight of tops was considerably greater in the shade in the clay soil; in the other soils few or no differences were found.

In July the roots extended downward about 19 inches in both the clay and loess soil, and 13 inches in the alluvial soil. In the shade they were not so deep. There were relatively few major laterals, the chief feature being a strong taproot. By September those in the open had penetrated to depths of 28 and 39 inches in the clay and loess soils, and to 23 inches in the alluvial soil. Little increase in lateral spread occurred throughout the summer.

*Quercus maxima*

SEEDLINGS.—Unlike the other species, red oak seedlings made their best growth in the clay and their poorest in the loess soil. Shading caused a marked increase in development of tops in both the clay and alluvial soils. Growth was very poor in the loess soil and tops were about equally developed in sun and shade (table V). Leaf area was 75 per cent greater in the shade at the clay and alluvial plots. Dry weight of tops was also greater.

Roots were most extensive in the clay soil and least in the loess at the time of the midsummer excavation. Laterals were about equally

developed in all three soils and under both conditions of light. Trees in full insolation at the clay and loess stations had a better development of laterals in the fall at which time they had reached depths of about 3.2 feet. Those in the shade were nearly as deep. Both sun and shade forms in the loess soil were less extensive, reaching only a little beyond the 2-foot level.

SAPLINGS.—Six saplings 5 to 8 years old and ranging in height from 3 to 12 feet were excavated. All grew on the lower slopes in clay soil and were shaded a part of each day. The strong taproots ranged

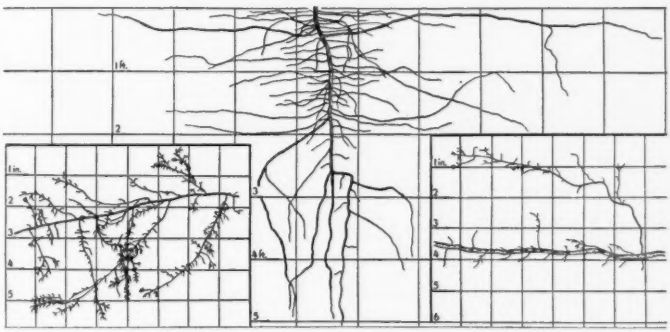

FIG. 10.—Root system of red oak in June of its fifth summer

in diameter from 0.85 to 2 inches. They tapered gradually; one 1 inch in diameter, for example, was 0.1 inch thick at 4 feet in depth and ended at 5.1 feet. A larger one extended to the 6.3-foot level. The course of the taproot in all cases was irregular, deviating 7 to 12 inches from the perpendicular. The irregular course and asymmetrical nature of the root system resulted no doubt from the hard clay soil.

The taproot was furnished with many rather horizontal branches, some of which spread widely, especially in the surface soil. Only a few of those in the surface 2 feet grew downward obliquely, but most of the roots below this level ran obliquely or nearly directly downward (fig. 10). Sixteen small branches, ranging from threadlike width to a thickness of 0.5 inch, arose from the first 6 inches of the taproot of a 5-year-old tree and 36 branches from one 7 years of age.

They varied in length from 1 to 5.7 feet. Nearly all had their entire course in the surface 6 inches of soil. Seven and 16 similar roots occurred on the second 6 inches of the two taproots, respectively, and 21 and 28 on the second foot; but branches below the 2-foot level were relatively sparse. Some of these penetrated as deeply as the taproots. Arising from this framework numerous branches thoroughly occupied the soil. The secondary laterals occurred at an average rate of 6 per inch but a maximum of 40 per inch was found. Smaller branches were also numerous, especially in the surface 2 feet of soil. Branching of roots of all sizes decreased about half below 2 feet.

*Hicoria ovata*

SEEDLINGS.—Seeds of shellbark hickory planted in the alluvial soil decayed during the wet spring. Growth in the other soils was so slow that by September the seedlings were only 4–5 inches tall (tables IV, V). The roots penetrated directly downward and their extent, as compared with that of the tops, was very great. By midsummer all the taproots had penetrated well into the second foot of soil, but in September those in the open at the loess plot were nearly 4 feet deep while those in the shade extended to 1.8 feet. In the clay soil both sun and shade forms were about 2.4 feet deep. Laterals were numerous, mostly horizontal, short, and poorly branched.

SAPLINGS.—Three trees 4–8 years old and 3–6 feet tall were excavated in upland clay soil, and one 4 years old and 4.5 feet tall in alluvial soil near a creek. All were characterized by an increase in the diameter of the taproot over that of the base of the tree in the first foot of soil, by a generally vertically downward course, a gradual tapering of the taproot, and a rather deep penetration. While the base of the tree in figure 11 was only 1.5 inches in diameter, the taproot was 1.8 inches thick in the first foot, 1 inch in the second foot, and 0.5 inch in the third foot. Except for one striking curvature it grew vertically downward, to a depth of 5.8 feet.

On all of the taproots laterals were sparse and small in the first few inches. The four to seven that did occur were relatively short and horizontal, but all were furnished with fine absorbing rootlets that extended to within 0.5 inch of the soil surface. The bulk of the

lateral roots, usually 13 to 18, arose between 6 and 17 inches' depth. Some were more than 0.5 inch in diameter. Most of the laterals arising in the first 15 inches of soil ascended somewhat and extended far outward, some to within 6 inches of the soil surface. Laterals over 5 feet long were found at this depth and others 6.3 feet long at a depth of 14 inches. All of these roots branched freely in the rich moist soil, as many as 20 branches per inch being common. They rebranched in such a manner that the absorbing area in the surface 15 inches was very extensive.

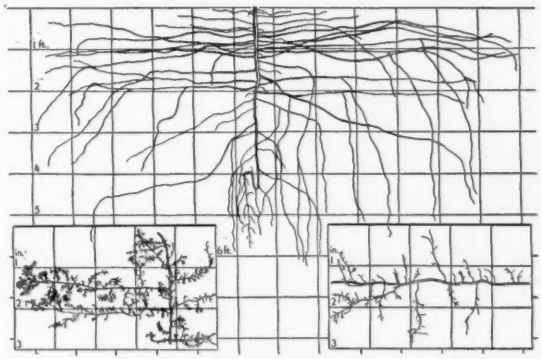

FIG. 11.—Root system of 8-year-old shellbark hickory in clay soil

With few exceptions, roots arising between the 15- and 24-inch levels extended downward. Some grew slightly downward after extending outward from the taproot 4 to 5 feet; others turned more directly downward, penetrating to an equal or even greater depth than the taproot, but rarely exceeding 6 feet. From the larger branches arising in the second foot many sinkers arose. These sometimes reached depths of 5 to 6 feet. The deepest one-third of the root system was relatively poorly branched. Thus the root system usually exceeded the top not only in length but also had about seven times as great a lateral spread.

### *Acer saccharum*

SEEDLINGS.—The hard maple seedlings had great difficulty in forcing their large cotyledons through the soil. They grew very

slowly both above and below ground. In the clay soil they reached 2.2 inches in height in the open but were twice as tall in the shade. In the alluvial soil they were about 2 inches tall under both intensities of light. The number of leaves and total leaf area in the clay soil were about twice as great in the shade but in the alluvial soil they were almost equal (tables IV, V).

The taproots by midsummer were still within the first 6 inches of soil. In the fall those in the clay were nearly 24 inches deep but in the alluvial soil they were still within the first foot (fig. 12). In the clay, root development in the shade exceeded that in the open.

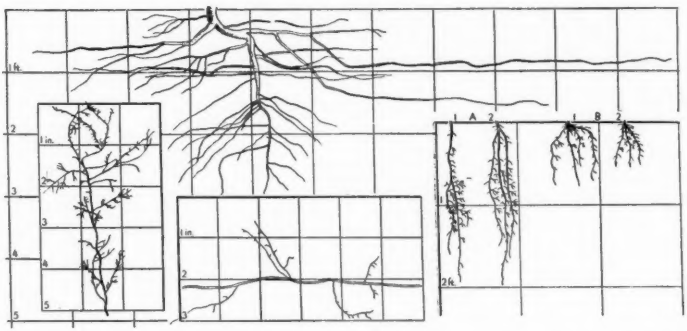


FIG. 12.—Year-old seedlings of hard maple (right) grown in clay (*A*) and alluvial (*B*) soils; root system of hard maple 16 years old (upper); and portions taken from the 6-inch level (left) and at 3 feet (lower center).

SAPLINGS.—Three trees, 8, 10, and 16 years old, growing on a gentle slope in clay soil were excavated. They were shaded nearly all day by a mixed deciduous forest so that height (5–11 feet) did not correlate with age. Although the taproots were 1–1.8 inches in diameter, they at once gave rise to such large laterals that their size was quickly reduced. As shown in figure 12, their course was not vertically downward. All grew obliquely downward in part, and the greatest depth of penetration was 3 feet.

Several of the shallower primary laterals on each taproot were 0.5 to 1.25 inches thick. Their general course was somewhat parallel with the soil surface, but they extended outward only 1.5–3 feet. Some of their branches, however, after descending to deeper levels

spread horizontally 5 to 7 feet. In the second and third foot branches were fairly numerous. They pursued either a horizontal or an obliquely downward course. Small rootlets were sparse, averaging near the soil surface about four per inch of main root. In the deeper soil they were even fewer and often the larger roots were bare for several inches.

*Quercus macrocarpa*

SAPLINGS.—Three 8-year-old trees of bur oak 1.5–3.5 inches in diameter and 7–12 feet tall were examined. They were growing widely spaced in upland clay soil. The strong, deeply penetrating taproots tapered gradually, in one case from 2 inches at its origin to 1.25 inches at 4 feet, to 0.25 inch at 6 feet, and to 0.12 inch at 10 feet. It tapered gradually to its end at 14.6 feet. The course was usually straight downward. Branches were numerous, frequently 18 to 24 arising from the first 14 inches of taproot. The largest were about 0.25 inch in diameter. The general direction of growth paralleled the soil surface although some roots extended upward and a few obliquely downward, some penetrating deeply. Between 14 and 24 inches numerous primary laterals 0.5–1 inch in diameter originated. These usually ran far outward, one to 11 feet. Sinkers arose from these at various intervals and penetrated directly downward to a depth of 9–12 feet. These sinkers branched freely, greatly increasing the absorbing area (fig. 13). Laterals arising at a depth greater than 2 feet extended obliquely downward, spreading only a little from the taproot and sometimes nearly equaling it in depth. The root system was well furnished with small laterals which had small tertiary branches with their sublaterals rather uniformly distributed throughout.

*Populus sargentii*

SAPLINGS.—Four saplings of cottonwood 3–7 years old and 2.5–13.5 feet high were excavated. Two were growing in a loam soil underlain with clay, and two were in clay soil. In all cases the deep taproot tapered gradually. On the largest tree, for example, it was 2.7 inches thick at the soil surface and 1 inch in diameter at 4 feet. All but one penetrated almost directly downward and reached depths varying from 5 to about 8.5 feet. One taproot divided dichotomously

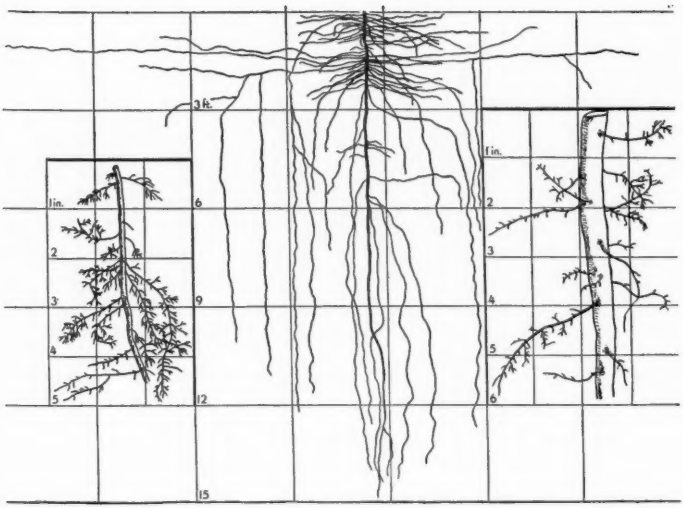


FIG. 13.—Root system of 8-year-old bur oak in upland clay soil. Note the sinkers which extend deeply and increase the absorbing system.

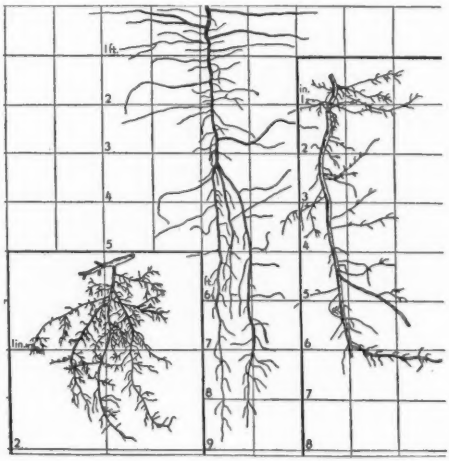


FIG. 14.—Root system of 3-year-old cottonwood

at about 3 feet, but the branches diverged only slightly from the perpendicular in their downward course (fig. 14). In the hard clay the taproots were neither smooth nor straight.

The taproots gave rise in the first 3 feet of soil to many strong laterals, some 0.5–0.75 inch in diameter, and a larger number of smaller ones. They all pursued a more or less horizontal course outward (some turning upward) to distances of a few inches to about 5 feet. Nearly all branched freely. The deeper portion of the taproot was also well supplied with primary laterals, but these were shorter and showed a strong tendency to extend obliquely downward.

Small rootlets occurred in great abundance near the soil surface, the finer ones at the rate of about 18 per inch. At greater depths these decreased in number and were more threadlike.

### Effect of shading on transpiration

Transpiration rates of boxelder, sycamore, buckeye, red oak, and hard maple were ascertained in full insolation and half shade. The plants were grown from seeds planted in the spring in containers 5 inches in diameter and 12 inches deep. Each container had a sloping roof with a circular opening in the center 2 inches in diameter. An opening near the bottom assured proper aeration; this was sealed during the experiment. The containers were filled with surface clay soil and buried in the earth to within 1 inch of their tops. When the plants had reached a suitable size (in July), the containers were removed and sealed after thoroughly watering the plants. They were then weighed and replaced for a period of one week after which the weights were again determined. Leaf area was measured and transpiration calculated on the basis of water loss in grams per square inch per week.

Two series, consisting of six plants of each species in the open and six in the shade, were used; one from July 4 to 11 and one from July 12 to 19. High insolation was continuous during the first period. The maximum day temperatures averaged nearly 100° F. and the minimum humidities about 32 per cent. The amount of sunshine during the second period was about 90 per cent; temperatures averaged about 4° lower than for the first period, and humidity 3

per cent higher. Wind velocity was higher during the first period. Transpiration was consistently higher under full insolation (table VI).

Hard maple, one of the most tolerant species, transpired 2.5 times faster under full insolation during both periods. Red oak, also a shade enduring species, transpired 72 and 52 per cent faster, respectively, in the open during the two periods. Sycamore and boxelder, the least tolerant, lost on an average 20 and 43 per cent more

TABLE VI

AVERAGE WATER LOSS IN GRAMS PER SQUARE INCH OF LEAF AREA IN FULL INSOLATION AND IN HALF SHADE

| SPECIES | JULY 4–11 | JULY 12–19 | AVERAGE |
|---|---|---|---|
| Boxelder | 6.66 (5.24)* | 5.03 (2.93) | 5.85 (4.08) |
| Sycamore | 8.04 (6.82) | 7.95 (6.45) | 8.00 (6.64) |
| Buckeye | 7.24 (6.62) | 7.25 (5.25) | 7.25 (5.99) |
| Red oak | 8.38 (4.87) | 6.28 (4.16) | 7.33 (4.52) |
| Hard maple | 11.00 (4.46) | 11.08 (4.43) | 11.04 (4.45) |

* Water loss in half shade given in parentheses.

under full sunlight. Buckeye averaged 21 per cent more in the open. Losses during the first period were greater owing to higher temperatures, more sunshine, and stronger winds.

## Discussion

Examination of roots in midsummer showed that each species had a characteristic juvenile root habit. In agreement with the findings of TOUMEY (**25**), "the . . . . root system. . . . follows a definite course of development and maintains a characteristic form for a rather definite period of time following germination." Except for greater depth of penetration in the mellow loess soil, buckeye and shellbark hickory, which grew slowly, showed little modification under the different environments even by September. Differentiation in the honey locust was likewise largely a difference in the depth of penetration. Boxelder penetrated the loess soil twice as far as in the

clay and almost three times as far as in the alluvial soil. Sycamore showed considerable differences in root habit, branching most widely in the clay, and penetrating much farther in the loess. Differentiation in the red oak was marked, the juvenile form being retained longest in the loess soil; much branching and wide lateral spread were characteristic in the clay and alluvial soil. Walnut and hard maple showed the greatest differences in the several soil types. The largest branches of the walnut penetrated laterally far in the clay soil; in the loess they extended very deeply, somewhat parallel to the taproot. Roots of the hard maple penetrated over twice as far in the clay, where the strong branches paralleled the taproot; but in the alluvial soil the branches spread obliquely downward. Thus certain species respond more quickly than do others to changed conditions of water content, aeration, and other factors of the environment.

Species which germinate in upland soils in a relatively dry forest climate where drought in the surface layers is imminent must produce rapidly growing, deeply penetrating, and extensive initial root systems if they are to become permanently established. HOLCH (13), for example, found that the shade tolerant seedlings of *Tilia americana* could not survive in loess soil under full insolation in southeastern Nebraska, since the surface layers were depleted of their available moisture to a depth greater than that of the root system of this moisture demanding species.

The greater extent of root systems under full insolation was due largely to the fact that strong insolation promotes transpiration. The transpiration rates, while not so high as those determined by HOLCH (13) from deciduous tree seedlings in southeastern Nebraska, compared favorably with the results of WEAVER and THIEL (28), who worked on the margin of the deciduous forest in Minnesota. In the honey locust alone was growth of tops greatly retarded by shade. HOLCH, growing seedlings in different forest sites, in some cases where light intensity was very low, found light to be of major importance. WEAVER and HIMMEL (26) have shown that light affects greatly the growth of plants both above and below ground. SHIRLEY (20) demonstrated that an increase of light intensity up to 20 per cent gave a proportionate increase in growth and that root

development was always poor under low light intensity. BURNS (4) and PEARSON (18) have obtained essentially similar results.

Growth in the wet soil of the floodplain was greatly retarded during early summer. Decreased development of tops was probably partially due also to the resulting decreased absorbing area of roots. DEAN (8) found that increased aeration produced uniformly greater development of both roots and tops. HOFFMAN and SCHLUBATIS (12), working with raspberries, ascertained that the height of the water level corresponded almost as closely as did the root distribution to the type of plant developed.

Distribution of trees probably depends largely upon the adaptability of the roots to the environment. Boxelder is notably a floodplain tree. Here it usually had a rather vertically descending taproot, with large, widely spreading laterals. When found in hard upland clay, the lateral roots penetrated deeply vertically and only a little in the horizontal direction. The much flattened and distorted roots indicated that the plants were not in their usual habitat. Cottonwood roots penetrated deeply in the upland soil but also had a well developed absorbing system near the surface. The great flexibility of the root system is shown by the fact that mature trees, growing in sandy soil along the Platte River where the water table is permanently high, are sometimes blown over by the wind. They then exhibit a "flat-bottomed" root system, the laterals extending widely but the depth of penetration being shallow.

Black walnut and honey locust are not only deeply rooted but also have a widely spreading root system, and consequently a large absorbing area. This enables them to thrive in fertile upland soil as well as on the floodplain. HOLCH (13) states that 3-year-old black walnut trees penetrated beyond a depth of 9 feet and that the laterals extended to a distance of more than 7 feet on all sides of the plant. Seedlings of honey locust less than 3 months old and only 8 inches high were found by CLEMENTS *et al.* (6) to possess a taproot system 40 inches deep and widely spreading horizontal branches 6 to 18 inches long.

Hickory, red oak, and hard maple, which normally grow on moist well drained slopes in Missouri, have generalized root systems which

penetrate, in the sapling stage, about equally in all directions. Bur oak, on the other hand, occurs under the most xeric conditions and penetrates more deeply and also more widely than the preceding species. HOLCH (**13**) found that the first year it reached a maximum depth of 5.7 feet and a spread of almost 3 feet. In addition, the bur oak is well supplied with sinkers which add to the efficiency of the absorbing system. WEAVER and KRAMER (**27**) found that bur oaks 50 to 65 years old have laterals extending outward 60 feet.

The roots of the trees examined made the greater part of their growth in the vertical direction during the first two or three years. Later they developed widely spreading laterals. This is especially well illustrated by the root habits of the bur oak which are now known from the seedling stages to maturity (**13, 27**).

## Summary

1. Half shade favored the growth of seedlings of black walnut, buckeye, red oak, shellbark hickory, and hard maple but retarded growth of honey locust, boxelder, and sycamore.

2. Root systems were deeper and more branched in all cases where the seedlings grew in full insolation.

3. Transpiration rates were consistently higher in full insolation. Hard maple transpired 2.5 times more rapidly but boxelder only 20 per cent more than in the shade; other species were intermediate.

4. The root systems of seedling honey locusts were 1.5 times as deep as the height of the top, and those of shellbark hickory 10 times as deep; other species were intermediate.

5. Root penetration of all seedlings, except red oak, was greatest in loess soil, where depths of 36 to 65 inches were attained.

6. Total lateral spread of seedling roots varied from 6 to 18 inches, except those of black walnut which spread 4 feet in clay, 3 feet in alluvial soil, and 3.5 feet in loess.

7. Roots were most poorly developed in the alluvial soil by midsummer, owing to deficient aeration in spring; by September honey locust and black walnut had penetrated deeper here than in the clay soil.

8. Honey locust has a generalized root system readily modified by

environment. In upland soil taproots of saplings penetrated to 5 feet, but on the floodplain they penetrated only to 2 feet, and laterals extended outward 10 to 17 feet.

9. The generalized root system of boxelder is very plastic. On the upland the taproot and nearly all the major branches penetrate deeply. In alluvial soil the root system is much shallower but extends widely in the surface foot.

10. The taproots of 6-year-old sycamore saplings penetrated upland soil to about 7 feet, branched widely at all depths, and were also furnished abundantly with deeply penetrating laterals.

11. Seven- to 12-year-old black walnut saplings developed strong taproots 4–6 feet long with many oblique branches spreading 3–6 feet and often reaching similar depths. In addition numerous strong horizontal laterals extended outward to distances two or three times the diameter of the crown.

12. The root systems of 5- to 8-year-old red oaks were deep, abundantly supplied with mostly horizontal laterals in their upper course and obliquely descending ones at greater depths. A column of soil 9 feet square and 5 feet deep was usually well occupied and a few strong branches extended 6 or more feet laterally.

13. The widely spreading root system of shellbark hickory thoroughly occupied the soil within a radius of 6 feet from the tree and to a depth of 5 feet when only 6 to 8 years old.

14. Hard maple has a relatively shallow root system, that of a 10- to 16-year-old sapling extending to depths of only 2.5 to 3 feet. Large laterals were fairly numerous; a few spread beyond 3 feet, but the smaller branches were relatively few.

15. The sturdy taproot of bur oak penetrates upland clay to 15 feet in 8 years. Laterals from the upper portion were abundant and a few spread far outward and in addition gave rise to sinkers that extended deeply. Deeper laterals usually pursued an obliquely downward course.

16. The shallow rooted cottonwood of the floodplains sends its strong taproot deeply in upland soil, at the rate of 1 foot or more each year. It is well furnished with laterals throughout but these do not spread widely for 6 or more years.

Grateful acknowledgment is made to Dr. J. E. WEAVER for outlining the problem and for direction and encouragement throughout the course of the investigation.

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# PHYSIOLOGY OF PINES INFESTED WITH BARK BEETLES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 460

RALPH W. CAIRD

(WITH ELEVEN FIGURES)

## Introduction

The great loss of timber in the southern states due to infestation by the southern pine beetle (*Dendroctonus frontalis* Zimm.) has necessitated the search for better means of control of this insect. This physiological study was made to obtain some clue as to the factors involved in the death of the trees. The situation is complicated by the penetration of the wood by various fungi which are always associated with the beetle attack (**4, 5**).

These studies are a part of the program conducted by the United States Bureau of Entomology on the Bent Creek Experimental Forest near Asheville, North Carolina. The work was done in the laboratories of the Appalachian Forest Experiment Station of the U.S. Forest Service under the direction of Dr. F. C. CRAIGHEAD. The scope of the work was greatly increased by the assistance given by Dr. W. C. BRAMBLE, of the Bureau of Plant Industry, and the loan of equipment by the botany department of the University of Chicago and the Bureau of Plant Industry.

## Descriptive studies

### METHODS FOR MOISTURE ANALYSIS

*Dendroctonus frontalis* is a primary bark beetle, capable of infesting healthy pines and maturing its brood. Severe outbreaks have been common in the Virginias and Carolinas, Tennessee, Georgia, Alabama, Mississippi, Louisiana, and Arkansas. It is especially destructive to *Pinus taeda*, *P. echinata*, and *P. virginiana* (**6**).

*D. frontalis* outbreaks usually center about a pine or group of pines which have been injured in some way by lightning, drought, girdling,

defoliation, fire, etc. To induce beetle attacks in the stand chosen for this study, cages of wire netting filled with bark containing beetles about to emerge were set up around the bases of scattered trees. As the beetles left the bark and attacked the inclosed portion of the tree, other beetles in the vicinity were attracted to the caged trees and the neighboring healthy ones. In this way it was possible to select uninfested trees and to keep a record of the dates of attack. Certain induced outbreaks furnished trees in the early stages of attack and others gave the later stages.

The studies reported in this paper were limited to a site of sandy clay soil about two acres in extent, which afforded a pure stand of *Pinus echinata* Mill. making rapid volume and height growth. Only dominant and co-dominant trees ranging in age from 20 to 25 years were used. These were 27 to 32 feet in height and were unbranched up to about 17 feet. Their diameter at breast height was from 4 to 6 inches and no heart wood had formed.

Many *D. frontalis* beetles simultaneously attack the tree at about mid-stem. The lower and upper portions of the trunk are usually infested a day or two later. Within three or four days after the mid-stem is infested, *Ips avulsus* Eich. is attracted to the injured tree and bores into the bark from the upper limit of the *D. frontalis* attack (20 feet) up to the leader. The two beetles cause similar injury, and in most cases are the only bark beetles involved. Usually the bark is perforated by the entrance holes from near the ground line to the leader. The holes bored through the bark are small. The size of *D. frontalis* is only about 3 mm. in length and 1 mm. in width; *I. avulsus* is somewhat smaller. The insects bore through the corky bark and tunnel in the phloem, but do not enter the wood at any time (fig. 1). The flow of resin into the wounds soon stops and the greater part of the galleries appear free from resin. At intervals along the tunnels ventilation holes to the outside are made. The entire life cycle of the beetles is completed in from 30 to 40 days, three to five broods developing in a season (**6**).

It was necessary to place the tree in dye solution to note which rings were carrying the transpiration stream. A method for suspending the tree in position in order to place the base in the solution was devised by Mr. HUCKENPAHLER. Two 2×4″ timbers were nailed to

three trees in a line, one at 3 feet above the ground and the other at 12 feet (fig. 2). With the center tree supported in this manner, it was ready for the next step in the experiment. A section was removed from the tree by cutting the trunk through at 0.5 foot and again at 1.5 feet. After this section or disc was removed, a pail of

FIG. 1.—Beetle galleries and *Ceratostomella* (bluestain); tunneling in phloem by southern pine beetle and bluestain extending in vertical streaks from the galleries.

dye solution was inserted in the gap (fig. 2), adjusted so that the bottom of the suspended tree was immersed in the dye. A satisfactory dye solution contained 3 gm. of light green in 6 quarts of water.

Entrance of air into the tracheids was partially avoided by making the lower cut first and by replenishing the dye solution when necessary. The solution was often prevented from rising more than 3 or 4 feet the first day by the accumulation of resin on the cut surface. This difficulty was overcome by sawing off a disc about 1 inch

thick from the bottom of the suspended trunk each morning and evening for 3 days.

After the base of the trunk had been in the dye solution for 3 days (70 hours), the tree was taken down, limbed, and the trunk notched at 3-foot intervals in a straight line from the butt to the leader. A disc 2 inches thick for mapping and for moisture samples was removed at each notch. Diagrams showing the distribution of the dye and any discoloration due to *Ceratostomella* spp. were made by placing tracing paper over the face of the disc (fig. 3 *A*). The annual ring was used as the unit for sampling, rather than an arbitrarily measured depth. The tissues were grouped for moisture sampling as follows, beginning with the outside: phloem, first three rings (1–3 inclusive); second three rings (4–6); third three rings (7–9); any remaining rings in the center of the tree, called the "center" (fig. 3 *B*). The samples for moisture determination of the phloem were secured by removing the corky bark with a knife and stripping off the phloem. The wood for moisture determination was chipped off with a knife. The knife blade was placed next to the summerwood limiting the ring group (heavy black lines in fig. 3 *B*) and the wood chipped off by striking on the back of the knife. The fine chips lost water rapidly and it was found that the error involved was less if the weight of the sample was determined by weighing the disc before and after chipping rather than by weighing the chips themselves.

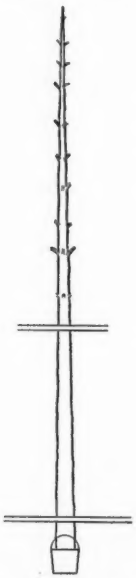

FIG. 2.—Method of suspending tree to place it in dye solution.

The samples were dried in paper bags in a large Freas electric oven equipped with a fan and regulated to 100° C ± 1°. Heating was continued until none of nine heavy samples from different parts of the oven lost more than 0.04 gm. after 8 hours of continuous heating between weighings. The wet weight of the samples was usually about 35 gm. The average error due to various causes was probably well within 5 per cent of the true value.

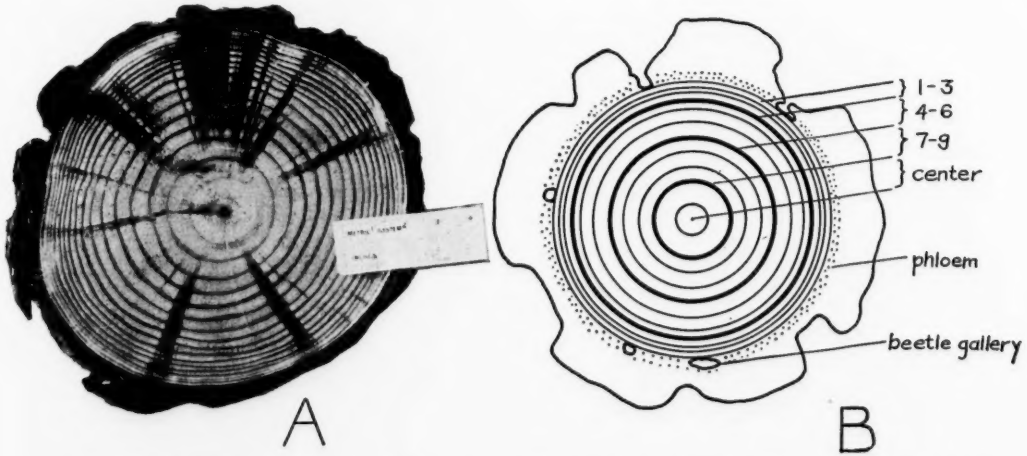


FIG. 3.—*A*, cross section of wood infested with *Dendroctonus frontalis* 30 days after attack (width of annual rings shown on rule); *B*, groups of rings taken to make composite samples for determination of moisture content.

### THE HEALTHY TREE

The conduction of dye solutions and the distribution of moisture were determined for the healthy tree in order to form a basis of comparison with the diseased tree.

DISTRIBUTION OF DYE SOLUTIONS.—The general distribution of dye in a healthy tree is shown in figure 6 *A*. The main course of the dye solution is up the outer rings. It passes up the inner rings more slowly, and if the tree is allowed to remain in the solution all of the rings except the center ones become colored. Vertical conduction appears to be mainly up the springwood. The dye solution which first enters reaches only 3 or 4 feet during the first 24 hours, owing to the accumulation of resin on the cut surface. During the second 24 hours, after removing a section of the base to secure a clear surface, the dye may reach mid-stem or under some conditions the branches and leaves. Irregular one-sided distribution is often observed, although more frequently the distribution is uniform. The period of three days is probably sufficient for the dye to travel as far as it will in the outer layers of the healthy and diseased trees. Distribution may be irregular during the first two days, but on the third day the outer rings are uniformly dyed. As the top of the tree is approached the dye is confined to the outer one or two rings.

It is assumed in this study, without actual proof, that the lateral movement of the dye is the same as that of the original solution. The dye may diffuse into cells which are full of sap without any movement of the water which carried the dye up the trunk. The water undoubtedly advances up the stem more rapidly than does the dye, since the wood absorbs a certain amount of the latter.

MOISTURE GRADIENTS.—The moisture content of the various layers is not the same at each height up the tree, but presents definite trends or gradients from the base to the top, as indicated in figures 4 *A* and 6 *A*. An actual tree is represented, and although it is somewhat more regular than the usual healthy tree, it shows the general relationships very well. The outer three rings have the highest moisture content of the wood samples, the values averaging from about 110 per cent (dry weight basis) at the base to 175 per cent at the top. These higher values are probably due to the immature cells

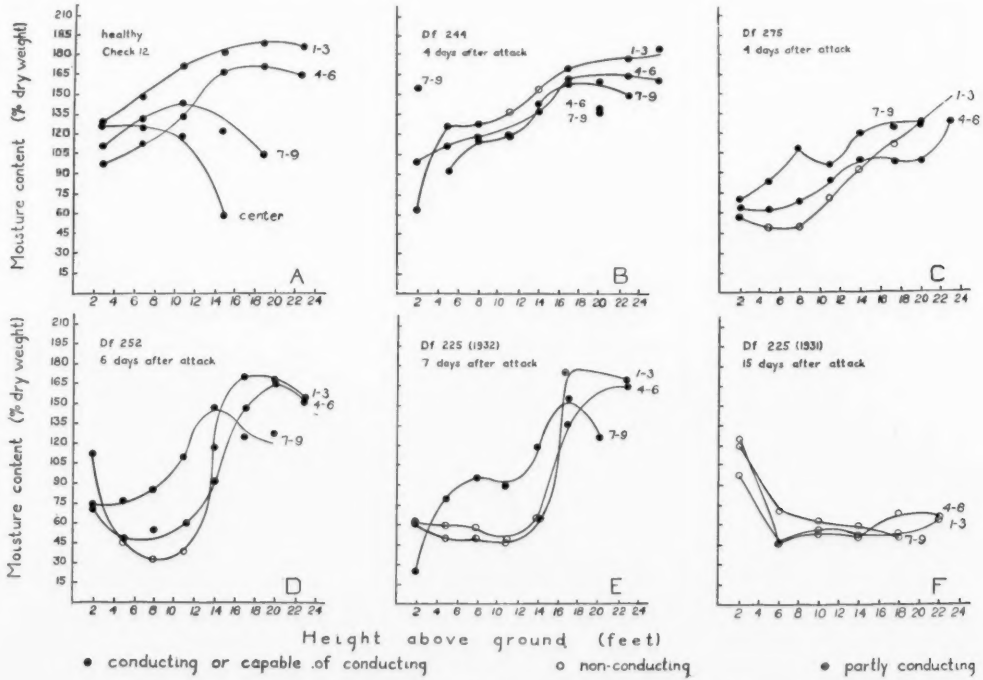


FIG. 4.—Successive stages in drying of tree and loss of ability to conduct dye solutions

adjoining the cambium and to the water of the transpiration stream. Rings 4–6 have a lower moisture percentage than rings 1–3, but have a similar type of curve. The remaining ring groups have intermediate values at the base which result in a crossing of the curves. In the top of the tree the values of all the groups are widely separated, a sharp drop in moisture content being frequently observed.

The reasons for the moisture gradients have not been investigated experimentally. The annual rings of these trees are narrow at the base of the tree and wide at the top, which probably results in denser wood at the base, leading to a lower moisture percentage. The sharp drop in the moisture content of a given group of rings when the top of the tree is approached may be the result of a functional change as these rings become the center of the tree. For example, at the base of the tree the fourth ring probably carries a portion of the transpiration stream, a function which it loses farther up the trunk. The rather slow passage of the dye solution up the center of the tree and its more rapid movement up the outer rings indicate the possibility of a gradual shift in the transpiration stream from many layers at the base to only one or two outside layers at the top of the tree.

The wide range of values obtained for the trees as indicated in figure 5 (*A*, *B*, *C*) is partly due to variations in height, diameter, and crown development, as well as to the introduction of a dye solution. Any dominant or co-dominant tree, picked at random from the stand, could be expected to have the typical graph form of the healthy tree, and to have the values fall within fairly definite limits. The diseased trees, as will be seen later, have a distinct graph form which varies significantly from that of the healthy trees. Sufficient work has been done with trees not steeped in solutions to indicate that the moisture relationships are general for the tree as it occurs in the field.

## THE DISEASED TREE

The changes occurring when the beetles infest the healthy tree are marked. The outer rings fail to conduct dye solutions, drying takes place in these outer rings, and various fungi enter the wood.

DISTRIBUTION OF DYE SOLUTIONS.—No apparent difference from the healthy tree can be noted in the general distribution of dye in

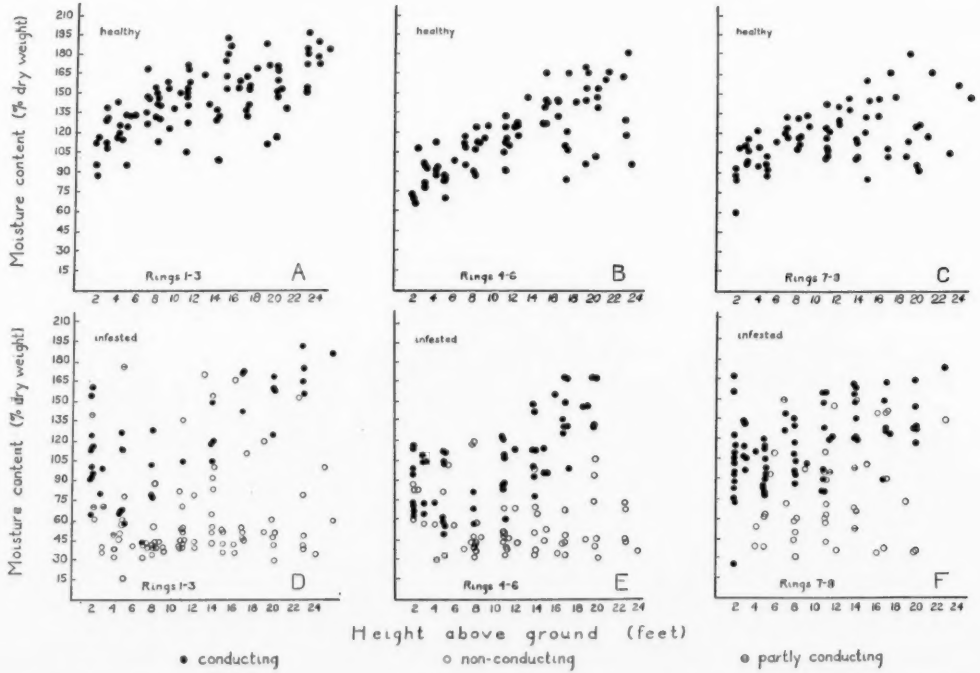


FIG. 5.—Moisture content of healthy trees as compared with trees infested with bark beetles. Percentage of moisture in wood of infested trees drops below that of healthy trees. Wood containing less than 75% moisture is incapable of conducting dye solutions.

the diseased tree during the first, second, or third days after the beetles' attack. The dye passes up to the leaves in the outer rings. Examination during the second and third days shows light colored patches in the wood along beetle galleries, caused by the accumulation of air in the cells as drying takes place. The first annual ring or two become non-conducting on about the fourth day after attack. As the outer rings become non-conducting, the transpiration stream is carried in rings which are closer and closer to the center of the tree (figs. 6, 8). In the early stages of attack the position of the dye in the top of the tree is the same as in the healthy tree. In later stages of the disease no dye reaches the top, owing to the stoppage of conduction at mid-stem.

DRYING OF WOOD.—The moisture content of the diseased tree soon falls below that of the healthy tree (fig. 4). The phloem becomes brown and commonly its moisture content falls from 225 to 100 per cent (dry weight basis). Rings 1–3 dry and become non-conducting before rings 4–6, and the latter before rings 7–9; that is, the drying progresses from the surface of the wood toward the center. Drying does not continue indefinitely but reaches a minimum moisture content at about 30 per cent. This lower limit is reached first by rings 1–3 at mid-stem. After 25 to 30 days the entire trunk is usually dry except for the base (figs. 4 *F*, 6 *F*). During subsequent decay the tree may become moist again, but such changes are outside this study.

Drying of the wood is correlated with loss of ability to conduct dye solutions, as is indicated in figures 4 and 5. As the wood dries it passes from a conducting to a non-conducting condition. The graphs, which contain all available data, serve to indicate a zone of demarcation lying between the wholly conducting and the wholly non-conducting values. This transition occurs in wood containing 65 to 85 per cent moisture (dry weight basis), as compared with the healthy tree values of from 100 to 185 per cent. This is a percentage figure, of course, and does not give the value of a single tracheid. The values presented are the sampling points in which the indicator dye was actually present in the wood.

FUNGAL PENETRATION.—The wounding of the tree by the beetles and the drying of the wood might be considered as the causes of the

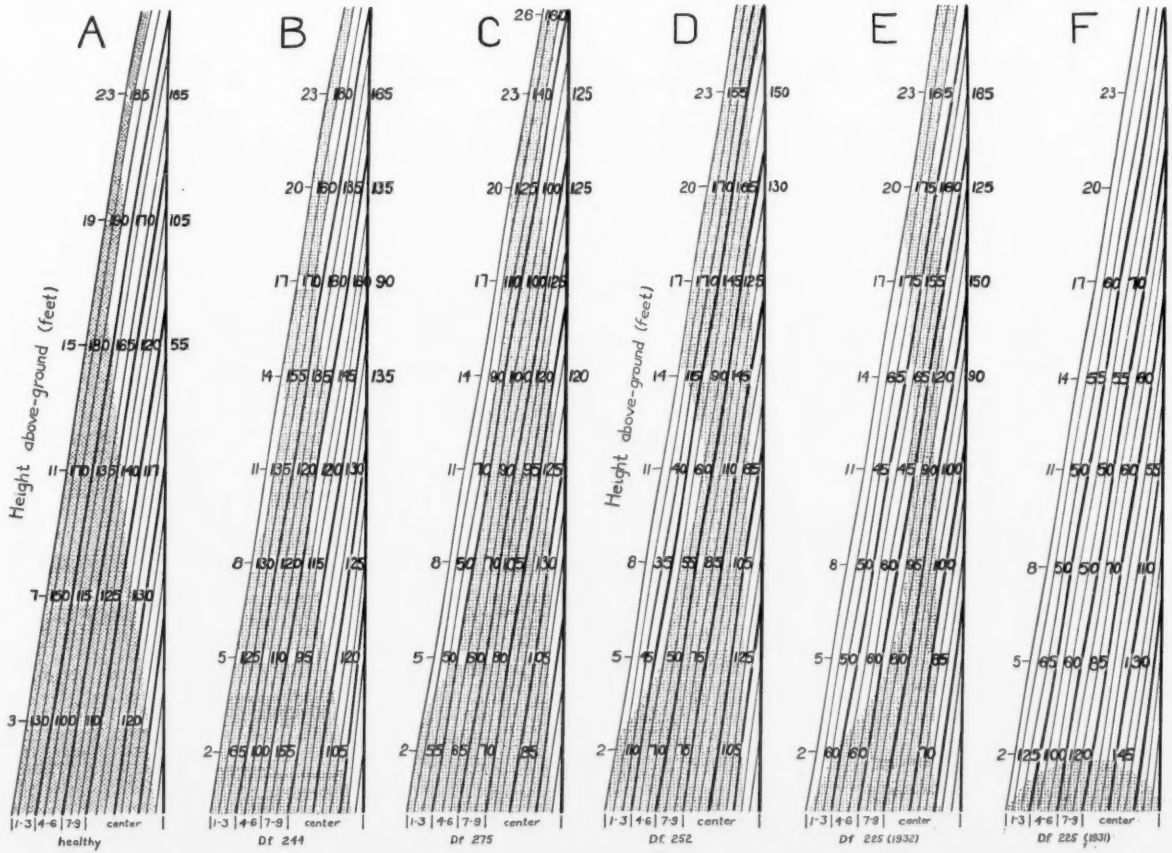


FIG. 6.—Loss of ability to conduct dye solutions as trunk dries. Moisture contents in heavy figures are from graphs in fig. 4

stoppage of conduction, but it will be seen from the following description of the penetration of fungi into the tree that the action of the fungi might in itself account for the death of the tree. Although it seems more probable that these various factors are interdependent, each must be examined as possible single causes. It was particularly desirable to determine what fungi penetrated the wood of the diseased tree and to establish the position of the fungi with reference to the ascending dye stream.

METHODS FOR FUNGAL ANALYSIS.—In preliminary work during the 1931 season thirteen trees attacked by beetles were sampled for fungi. The trees were sampled systematically at 3-foot intervals, 50 pieces of wood tissue, or plantings, being taken from each tree with about 700 plantings in all. Approximately 1000 cultures were handled. The sampling intervals chosen were too widely spaced and the data secured were insufficient for the purposes of the investigation. The work done during 1931 was improved upon in 1932 and the results are given in detail.

Trees representing progressive stages of the disease were placed in dye solution for three days before they were taken down and limbed. The bark was scored with a hand saw in a line from the base to the leader in order to orient the discs so that they might be arranged in the same position they occupied in the standing tree. The trunk was then cut into 1-inch cross sections which were numbered consecutively according to the inches above the ground. Enough discs were taken into the laboratory to satisfy the day's requirements and the remainder were stored in a refrigerator regulated to 5° C. Tests by Dr. BRAMBLE indicate that this temperature is sufficiently low to prevent appreciable growth of *Ceratostomella pini* Münch, molds, and bacteria and yeast, if they are not kept for more than five days. The length of time needed for sampling a tree was not more, and usually less, than three days.

Wood for fungal cultures was secured by cutting a block about 1 inch wide along a radius of the disc in such a way as to include all of the rings from the bark to the pith (fig. 7 *A*). After transferring the block to the inoculation chamber, the bark was removed and tissue taken from the springwood of each annual ring. The wood was split by placing a sterile scalpel along the springwood of the annual ring

which was to be sampled and striking down gently with another object. Care was taken to start the splitting with a sterile scalpel and then to pry with the scalpel to complete the splitting without touching the area from which the tissue was to be taken. A second sterile scalpel was used to cut off a thin slice of wood about 3×4 mm. in surface dimensions from the center of the exposed surface of the block. Five plantings each were taken from the first two rings, and

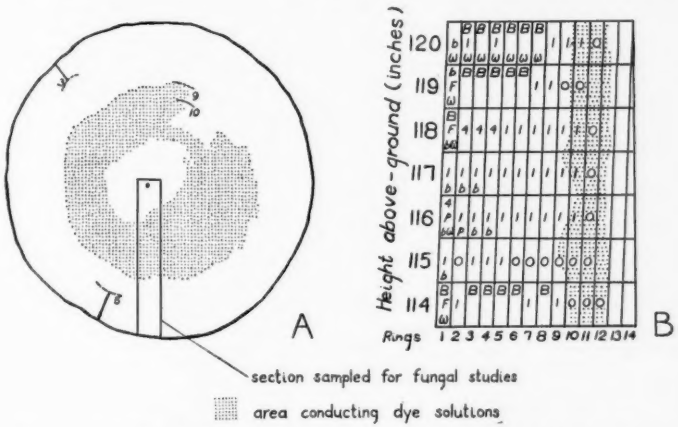


FIG. 7.—*A*, section of *D. frontalis* 355 at 120″ sampled for fungal studies. *Ceratostomella* apparent to the eye is indicated by heavy lines to the third and fifth rings. *B*, actual data collected for *D. frontalis* 355 at 114 to 120″. Key: *B*, *Ceratostomella* (bluestain); *1*, fungus no. 1; *w*, bluestain wedge observable in wood; *O*, negative cultures; *b*, bacteria and yeast.

placed on malt agar in petri dishes. One planting each was taken from the successive rings toward the center until a point two or three rings within the area dyed by the indicator solution was reached (fig. 7 *B*). The latter plantings were placed on malt agar slants in test tubes. The tissue was pressed into the agar so as to have a portion imbedded. The only culture medium used was malt agar, upon which most fungi which decay wood will show some growth. This was prepared from Bacto-malt-agar, according to the directions supplied by the Digestive Ferments Co. of Detroit, Michigan. Asheville city water was used. The pH of the medium was not adjusted and hence was approximately 5.5. A small amount of work was done

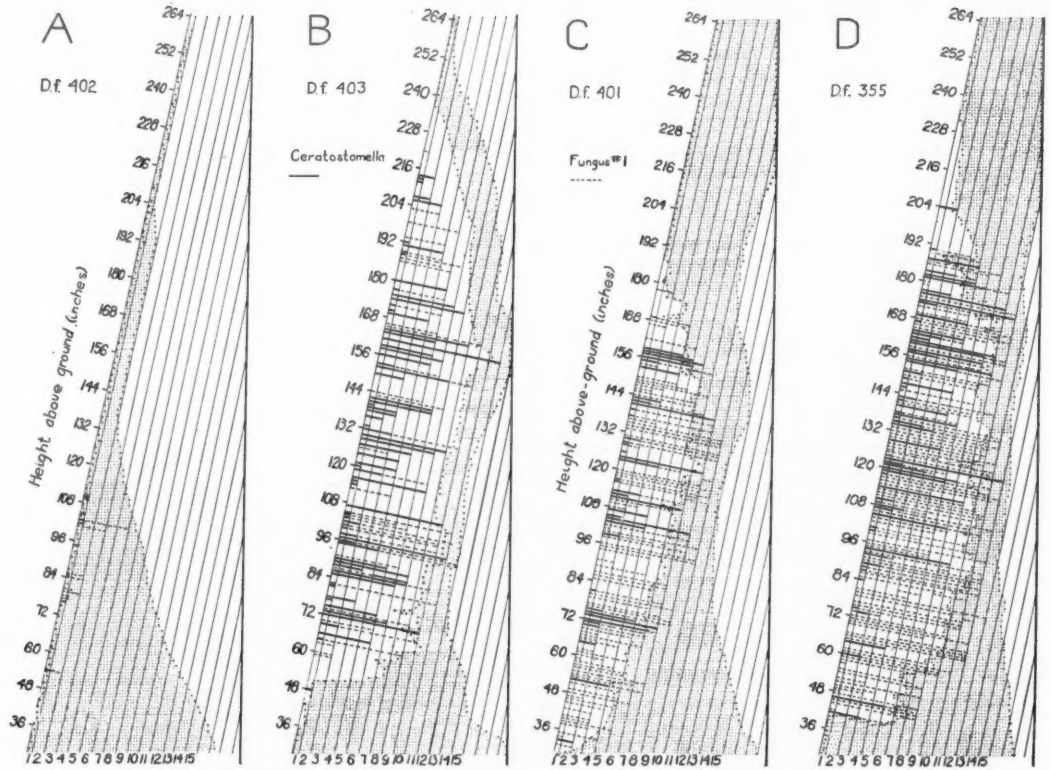


FIG. 8.—Penetration of *Ceratostomella* and fungus no. 1. Inclosed shaded area indicates wood conducting dye solutions. In *D. frontalis* 403 no samples were taken from 108 to 113″, 144 to 149″, or 180 to 186″.

with malt agar prepared in the laboratory from a popular brand of malt syrup, using 45 gm. of syrup to 1000 cc. of water and 20 gm. of agar.

Since the data collected by these methods lose their significance if there is no assurance that the fungi isolated were actually growing in the wood and were not merely contaminations, precautions were taken to reduce the number of contaminations as much as possible. Inoculation chambers were used, the cultures were stored in large museum jars, and the mold fungi destroyed without opening the containers. The cultures were kept from 10 to 14 days, which was longer than necessary for the fungi to show distinctive colonies. Cultures designated as "sterile" at the end of this time remained so if kept for four weeks or longer. The fungi were recognized by their habits of growth on the malt agar. The great majority of the cultures appeared to be of single fungi, but no attempt was made to isolate them as such. The fungi easily recognized were *Ceratostomella* spp., *Trichoderma lignorum*, *Penicillium* sp., *Aspergillus* sp., and "bacteria and yeast." The growth habit of fungus no. 1 is distinctive although the lack of fruiting bodies has prevented its identification. No description of the growth habits of this fungus has been published.

The *Trichoderma* cultures were identified as *T. lignorum* by Dr. BRAMBLE and Dr. RUMBOLD. A culture from the isolation study designated *Ceratostomella pini* by Dr. BRAMBLE was confirmed by Dr. RUMBOLD of the Forest Products Laboratory.

The chief error in designation of the fungi was probably the failure to observe fungus no. 1 when it occurred with *Ceratostomella* (fig. 7 *B*). Bacteria and yeast were occasionally missed when growing with higher fungi, and it is possible that yeast with hyphae were classified with higher fungi. These errors are probably negligible when the number of cultures examined and the use to which the data are put are considered.

Figure 7 shows the detailed results upon which the description is based. Figure 7 *A* shows the irregular distribution of the dye and figure 7 *B* gives a portion of the actual notes. In making up the graphs (fig. 8) the following rules were adopted:

In *D. frontalis* 403 no samples were taken from 108 to 113", 144 to 149", or 180 to 186".

1. Where fungus no. 1 occurs with *Ceratostomella*, only *Ceratostomella* is shown.

2. Where fungus no. 1 or *Ceratostomella* spp. were not found in some of the rings, the fungus is shown as present in all rings to the innermost one in which it is found.

3. Where notes are lacking as to dye conduction, the points where the data are available are connected with a smooth line.

FUNGAL STUDIES IN 1932.—The results of the 1932 study are given according to individual trees which represent different stages of the disease.

Tree no. 402 (fig. 8 *A*) represents an early stage of attack of *D. frontalis* about three or four days after infestation. The beetles were still extending their galleries and laying eggs. These beetles which first attack the tree and make the galleries along which the eggs are laid are designated parent-adults. The tree although only lightly attacked would no doubt have died if left standing. The indicator dye appeared in the outer ring along the line chosen for analysis except at four places. *Ceratostomella* was cultured in the first ring although not apparent to the eye. Fungus no. 1 was cultured in the dyed area of the fifth ring at 102 inches and had apparently penetrated into the wood more rapidly than *Ceratostomella*. Bacteria and yeast, *Penicillium*, and *Trichoderma* were found in the first ring.

In tree no. 403 (fig. 8 *B*) parent-adults, tiny larvae, and eggs of *D. frontalis* were present at mid-stem and *Ips avulsus* had entered the bark from the height of 14 up to 20 feet. Close correlation between the position of the dye and the position of the fungi was not expected, and exact notes as to the position of the dye for every inch were not always noted. The dye area shows indentations associated with the presence or absence of *Ceratostomella* or fungus no. 1. This is found in 24 instances where exact notes are available. These are the only fungi present to any great extent in the inner rings of the wood. *Ceratostomella* was secured in the first ring from approximately 40 per cent of the sampling points. From 52 to 63 inches the tissue appears to be sterile for the most part but not dyed. This may be due to the heavy tunneling of the bark by the beetles and the attend-

ant drying rather than to the *Ceratostomella* and the fungus no. 1 at 64 and 65 inches. It may be that in this tree the drying of the wood has advanced faster than the fungi, and that the drying may be acting alone, apart from any action of the fungi.

Tree no. 401 (fig. 8 *C*) had parent-adults and eggs present at the base, one-fourth to one-half grown larvae at mid-stem, and parent-adults, eggs, and tiny larvae at the top. Plantings to the number of 2520 were taken from 25 to 185 inches. The same relation between conducting (dyed) areas and non-conducting areas appears to hold. This tree is remarkable for the small amount of *Ceratostomella* found in the inner rings. It was frequently noted as being observable on the surface of the outer rings but was actually cultured in only 37 out of 150 sampling points. Fungus no. 1 was commonly found two or three rings within the dyed area and *Ceratostomella* in only four cases. The localized effect of bluestain is again seen in this tree.

Tree no. 355 had the parent-adults which had infested the tree about 6–8 days previously. Tiny larvae were in the base and mid-stem; eggs at 15 feet and higher. *Ips avulsus* had not as yet infested the top. The fungal diagram is based upon 3120 plantings taken at 1-inch intervals along the trunk from 36 to 204 inches. As shown in figure 8 *D*, the dye solution was taken up in the inner rings. The outer non-conducting rings were dry and almost solidly occupied by fungi. Fungus no. 1 was cultured from the conducting and partially conducting wood in a number of instances, while *Ceratostomella* was obtained in only two cases. The small amounts encountered from 40 to 90 inches make it seem improbable that this fungus was the cause of the stoppage of conduction observed. This area was dry and fungus no. 1 was obtained from it.

The brood of tree no. 400 was in the pupal and new-adult condition, about 30 days after infestation. The tree represents a very advanced stage of the disease, in which the leaves were yellowing and conduction up the stem had completely ceased. *Ips calligraphus* pupae were in the base up to about 25 inches, with *D. frontalis* above. The tree was sampled only from 8 to 60 inches. The wood was heavily "blued" by *Ceratostomella*, and this fungus was cultured from almost every planting. *Trichoderma* was also present in almost every

planting and quickly overran the agar. Bacteria and yeast were found even in the center of the tree. Other fungi were also present, but no attempt was made to separate the fungi in the mixtures.

### Discussion and summary of descriptive studies

It is obvious that an insufficient number of trees were analyzed to explore the conditions fully, although the main features are brought out.

1. *Ceratostomella* and fungus no. 1 are the only fungi which are present in considerable amounts in the interior of the diseased tree. From inoculation studies *C. pini* is known to be able to kill pines, but fungus no. 1 has not been tested in this respect.

2. The great development of *Ceratostomella* which appears as darkened wedges (fig. 3 *A*) does not show until late in the disease. *Ceratostomella* may penetrate the wood to the ninth or tenth rings and not be apparent to the eye.

3. *Trichoderma*, *Penicillium*, bacteria and yeast, and other fungi enter the inner wood after conduction up the trunk has been stopped. Some of the fungal forms encountered are no doubt the common fungi which rot fallen timber.

4. An unexpected, close relationship between the positions of the fungi and the outer edge of the conducting zone (dyed area) was found. A more exact study with refined technique is desirable, with careful microscopic examination of the tissue. The precise relationship between the conducting zone and the fungi cannot be drawn from this study, since the methods were not sufficiently refined and the probability of the growth of the fungi during analysis is present.

5. No moisture samples were taken of the trees examined for fungi in 1932, but the later moisture studies indicate that non-conducting zones are drier than conducting zones, and that even the outer edge of the conducting zone has lost moisture. The loss of moisture and the positions of the fungi are intimately connected; this study, of course, is merely descriptive and gives no method for establishing causal relationships. It is pointed out, however, that drying of the wood may occur without the immediate presence of any fungus, and that in this sense drying is a constant accompaniment of the stoppage of conduction but the presence (action) of fungi is not. *Cera-*

*tostomella* appears to have mainly a localized effect. It seems probable that the fungi act in some way to accelerate the rate at which the tree dries, and thus produce conditions favoring their growth.

6. The position of the fungi might be purely incidental to the stoppage of conduction, but from the inoculation studies it seems probable that the fungi are actually concerned in the death of the trees.

## Experimental evidence and general discussion

There is obviously need for experimental work to determine the action of the various factors involved when acting alone. This work is given briefly in this section, together with a discussion of the possible rôles of the various factors.

### Beetle galleries

The entrances to the beetle galleries and the extensive tunneling of the phloem serve to expose the surface of the wood to the outer atmosphere. Drying could not occur if the bark were not opened by the beetles. This is substantiated by an experiment in which the entire bole of a pine infested with *D. frontalis* was given a heavy coating of grafting wax applied after the bark had been smoothed. New ventilation holes made by the beetles were promptly filled. The trees used were comparable intermediates about 30 feet tall and 4 inches in diameter at breast height. The moisture relationships after 33 days are shown in figure 9 *A*. The beetle brood failed to develop, *Ceratostomella* did not penetrate, so far as could be observed, and the leaves remained green. In a repetition of the experiment in 1932 the trunks of the waxed trees dried out, but in each case beetles made numerous ventilation holes through the wax.

The water which is lost as the trunk dries may pass out through the leaves or through the beetle gallery entrances, or both. That at least some of the moisture may leave through the entrances was indicated by a further experiment in which the trees were topped. If the limbs are removed from a beetle-attacked tree the trunk dries out fairly rapidly even though the foliage has been removed. Three pitch pines (*P. rigida* Mill.) were used in the study, the results of which are given in figure 9 *B*. Two of the trees had been infested by *D. frontalis* for about three days and one was a healthy tree of com-

parable size. One of the infested trees was limbed and the other was not. The limbed tree lost water from the trunk as indicated by the moisture condition after 33 days. Some water may have been lost through the cut ends of the branches but on the repetition of the experiment in 1932, trees in which the ends were covered with grafting wax showed the same general relationships as those not covered. The drying of topped trees infested with beetles has been described by NELSON in the 1929 cooperative experiment (**2**).

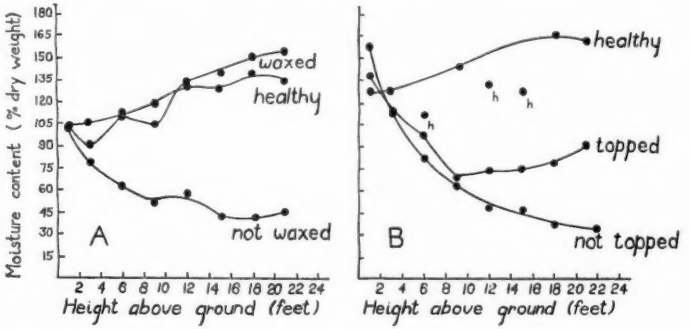


FIG. 9.—*A*, waxed-bole experiment: Tree infested with *D. frontalis* when waxed does not dry out to extent of a tree not waxed. *B*, topped tree experiment: Removal of limbs from tree infested with *D. frontalis* does not prevent drying out of trunk.

## NATURAL DRYING

If the bark is stripped from a healthy pine for a distance of even 10 feet up the trunk, the tree does not die until long after the tree attacked by the beetles. However, the outer cells of the wood become impregnated with resin. If the surface of the wood is kept relatively dry and free of liquid resin by rubbing it with powdered resin, the outer wood dries and becomes non-conducting for the outer four or five rings, but only after a long period of time. It is possible that if the experiment were repeated and a resin-free surface maintained, more rapid drying and death of the tree due to simple drying might result and the effect of drying without the action of fungi might be determined.

## Inoculations

The rôle of *Ceratostomella pini* and probably of fungus no. 1 and the other fungi appears to be the speeding up of the drying of the bole of the tree. There is a close relation between the position the fungi occupy in the tree and the drying of the wood. This physiological study was limited to the broader aspects of the disease, since the investigation of the effect of the fungi on the cells requires intensive study. From the knowledge that *C. pini* is limited largely to ray parenchyma (3), it is possible to visualize how the action of a fungus in penetrating cell walls and killing the living ray parenchyma might make easier the loss of water and the entrance of air into the tissue penetrated and into vertical elements. Throughout this study, the gases present in the cells in the dried wood are referred to as "air," without actual proof that this is true. This assumption is made from the observation of the gradual drying from the outside toward the center, and because there is no apparent suction or pressure in the dry wood on exposing dried trees to dye solutions.

When the tree is inoculated with *Ceratostomella pini* there is drying and attendant passage of the dye solution around the diseased zone which is strikingly similar to the behavior of the dye solution in the trees attacked by the beetle. This has been described by Nelson (2). Huckenpahler and Wygant inoculated four trees with pure cultures of *C. pini* and analyzed them to determine what changes had occurred with regard to dye conduction and moisture content. After 25 days the effect of a 3 inch band of inoculum (fig. 10 *A*) about the tree at 11 feet is as shown in the graphs of figure 11. Check trees, with comparable injury but without the fungus, were not available at the time, but later experiments showed that trees inoculated with *Trichoderma lignorum* and with sterile rice did not suffer a loss of moisture in the wood. A tree inoculated with *C. pini* and left standing had not died after one year, although it seems probable that the trees analyzed for moisture and fungi would have died. The *C. pini* was recovered from the wood by plantings but was not redetermined by an expert. Detailed moisture determinations were made of four trees in which the sharp drop in moisture content shown in figure 11 is repeated. The same type of drying occurs in trees in-

FIG. 10.—*A*, tree inoculated with *C. pini* in a 3 inch band at 12 feet. Inoculum applied as a poultice directly to wood by removing the bark which was then tacked back in place and the wound covered with grafting wax and waterproof cloth in an effort to prevent drying. *B*, tree killed by *C. pini* applied in a spiral band inoculation; tree protected from beetle attacks by wire screening and cloth.

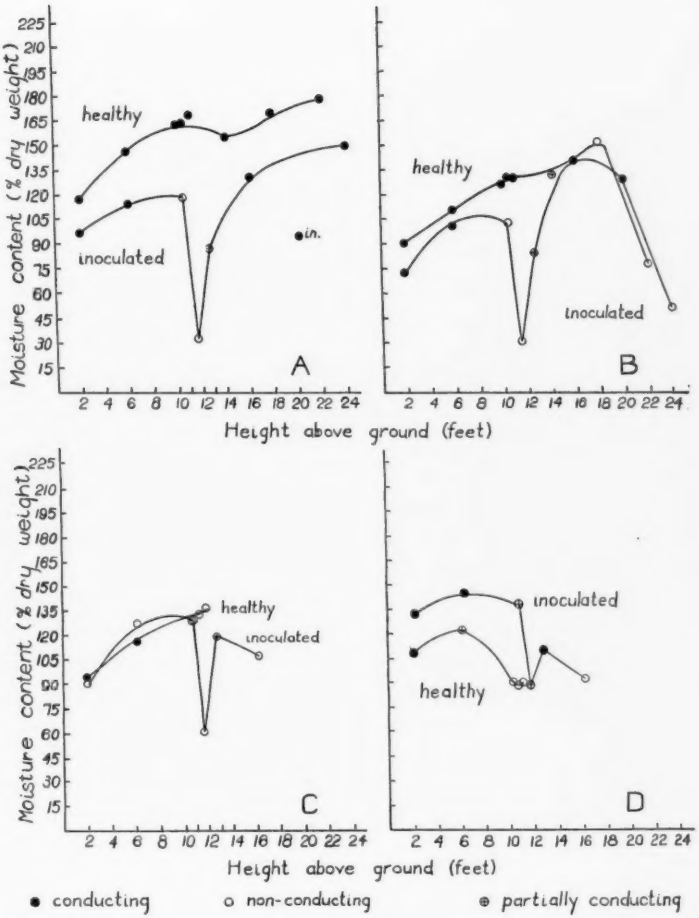


FIG. 11.—Inoculated tree no. 201 showing drying of wood at an inoculation band and loss of ability to conduct dye solutions. *C. pini* was recovered from the dried zone. *A*, rings 1–3; *B*, 4–6; *C*, 7–9; *D*, "center."

oculated with *Ceratostomella* in a spiral band (fig. 10 *B*), and in four bands at intervals of 2 feet. Later attempts to repeat the single band inoculations failed. The study of the tree infested with beetles, taken with the inoculation studies, serves to indicate that the death of the tree is due to the same cause in both instances. Future work might well make the inoculations a major study, since the factors can be isolated more easily.

## Summary

1. *Dendroctonus frontalis* and *Ips avulsus* bore through the bark and tunnel in the phloem. The beetle inoculates the wood with fungi and the entrance holes and beetle galleries serve to expose the wood to the air.

2. The outer rings of the tree lose the capacity to conduct dye solutions successively from the first ring toward the center. This gradually advances to the center of the tree and cuts off the supply of water to the leaves which empty the top of water and die.

3. Failure of the outer rings to conduct dye solutions is intimately associated with drying of the wood. The suggestion is made that the accumulation of air in the rings, which accompanies the loss of moisture, forms a resistance sufficient to stop the vertical and lateral transport of water.

4. The failure of the wood to conduct dye solutions is also closely associated with the penetration of the wood by fungi. *Ceratostomella pini* and fungus no. 1 are practically the only fungi which penetrate deeply into the wood during the early stages of attack. Inoculations show that *C. pini* growing alone is able to kill the trees, but fungus no. 1 has not been tested.

5. From the close connection between the drying and the position of the fungi in the wood, it is suggested that the drying is accelerated by the action of the fungi on the cells.

6. The stoppage of conduction is probably also associated with the aspiration of the tori, as indicated by NELSON's work. He suggests that blocking of the pits of the tracheids by the tori might account for the stoppage of conduction.

I have received suggestions from many with whom I have discussed the problem. Mr. R. A. ST. GEORGE and Drs. R. M. NELSON

and CARL HARTLEY have been especially helpful. The data were collected with the aid of field assistants during the summers of 1929 to 1932, inclusive, and the special contributions of these men are acknowledged in the report. Mr. N. D. WYGANT gave valuable assistance in the 1931 fungal work. The work was assisted by a Charles Lathrop Pack Forest Education Board grant, 1930 and 1932, while the writer was at the School of Forestry and Conservation of the University of Michigan.

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# MACROSPOROGENESIS AND DEVELOPMENT OF THE MACROGAMETOPHYTE OF SOLANUM TUBEROSUM

OLIVE L. REES-LEONARD

(WITH PLATES IX, X)

## Introduction

The production of fruits containing viable seeds is the exception rather than the rule in the potato. Extensive studies of microsporogenesis and of pollen development have been made to determine the causes of non-fruitfulness, but information concerning macrosporogenesis and the development of the embryo sac in *Solanum tuberosum* L. is limited. With the exception of the observations of YOUNG (21), apparently no detailed study of these latter phenomena in the potato has been made up to this time.

YOUNG made a brief study of the floral development, microsporogenesis, macrosporogenesis, macrogametophyte development, and degeneration of the sporogenous tissue in several varieties of *Solanum tuberosum*. After the archesporial cell has increased in size, he regards it as the macrospore. NANNETTI (16) concluded that the archesporium of *S. muricatum* develops directly into the embryo sac since no nuclear or cell divisions were observed that would indicate the presence of a tetrad of macrospores. BHADURI (1), in his study of *S. melongena*, traced the development of the normal 7-celled macrogametophyte which is derived from the functional chalazal spore in the linear tetrad of macrospores. KRÜGER (12) studied flower and fruit development, including macrosporogenesis and the macrogametophyte, in several members of the genus *Solanum*. She observed that in *S. nigrum* and in *S. tubingense* the micropylar macrospore of a linear tetrad is the functional spore from which develops a typical 7-celled macrogametophyte.

## Materials and methods

Under greenhouse conditions the buds of *Solanum tuberosum* abort early, so that it was necessary to collect material in the field. During

the flowering seasons of June, 1932 and 1933, entire flower clusters of the Irish Cobbler variety, containing buds of varied ages as well as mature flowers, were collected in the fields of Skinner's Nursery at Topeka, Kansas. In the latter part of July, 1933, material of the same variety was collected from the experimental plots of the Wisconsin Experiment Station. Flower clusters containing only small buds were placed in the fixing fluids intact. The tips of older buds were usually cut away to facilitate penetration. In cases of buds beginning to open and of mature flowers, the pistils were dissected out before being fixed.

The fixatives used were formal-acetic-alcohol (formaldehyde 5 cc., glacial acetic acid 5 cc., 70% alcohol 90 cc.), Flemming's medium solution, and Weinstein's modification of Licent's solution. The best results were obtained with formal-acetic-alcohol and with Weinstein's modification of Licent. Since the material tended to float on the surface of the solution, Carnoy's fluid containing chloroform was used for a few seconds as a "sinker," after which the material was transferred to the regular fixative to be used. After washing, the material was dehydrated, cleared in chloroform, and imbedded in paraffin.

Both longitudinal and cross sections were made at thicknesses varying from 5 to 18 $\mu$, depending upon the size of the buds. Sections 8 to 10 $\mu$ were most satisfactory for the study of young buds, but sections of mature pistils were cut 15 $\mu$ in thickness.

## Observations and discussion

### Development of ovule

The ovary of *Solanum tuberosum* consists of two united carpels, each with an enlarged placenta on which many ovules are borne. The ovule initials differentiate as groups of cells from the placental tissue when the bud is a few millimeters in length. As cell divisions occur in the epidermal and subepidermal layers, the ovule initials protrude and the placenta loses its smoothness of contour. Krüger (12) found that in *S. nigrum* only definite cell complexes in the subepidermal layers divide to function as ovule initials, which lie distributed over the placenta.

The integument of an ovule develops as a ring of meristematic

tissue, arising near the base of the nucellus (fig. 1) at a level immediately below that of the archesporial cell. This occurs during the resting stage of the archesporial nucleus or during pre-synizesis. In its early stages the integument may be three or four cells in thickness, but when fully developed it is usually five to eight cells thick. At the chalazal end of the ovule some of the cells of the short funiculus elongate and differentiate into a vascular trace, which connects with the vascular tissue of the placenta.

The nucellar tissue begins to degenerate soon after the meiotic divisions are completed. Accompanying its degeneration, the epidermal cells on the inner side of the integument become elongated perpendicularly to form a tapetum-like nutritive layer, the cells of which possess a dense cytoplasmic content. YOUNG (**21**) noted that the presence of this nutritive layer in the potato was continuous in the chalazal region with a group of angular cells against which the embryo sac rests. A similar nutritive layer was observed by SVENSSON (**20**) in *Hyoscyamus niger*. The ovule of *S. tuberosum* takes on a curved form as a result of uneven growth of its basal portion and of the enlargement of the integuments (figs. 1–4). The mature ovule appears to resemble the amphitropous rather than the anatropous or the campylotropous type. These latter types of ovules have been reported for many of the species of the Solanaceae investigated.

## MACROSPOROGENESIS

The single archesporial cell (fig. 2), which is usually present, is distinguished from the other cells of the nucellus by its larger size, denser protoplasmic contents, and by its greater affinity for stains. However, a multiple archesporium is frequently differentiated from hypodermal and subhypodermal cells (figs. 1, 3, 4, 7, 8). In one ovule, six archesporial cells of hypodermal and subhypodermal origin were observed. As previous observers have noted, an archesporial cell functions directly as a macrospore mother cell, not dividing to form a primary parietal cell and a primary sporogenous cell. Occasionally two, three, or four macrospore mother cells are found in early prophase stages of the heterotypic division (figs. 3, 4, 7). In the majority of the members of the Solanaceae which have been investigated, a single macrospore mother cell occurs. LESLEY (**13**), how-

ever, occasionally found two macrospore mother cells in an ovule of tomato. BHADURI (1) found that a multiple archesporium frequently occurs in *S. melongena*. Variations in the development of macrospore mother cells led him to conclude that "all the cells generally included under the group 'ovule initials' are potentially sporogenous."

In *S. tuberosum* there is usually but one archesporial cell which develops beyond the early heterotypic prophases. In several cases, however, two macrospore mother cells, which are in the early prophases of the heterotypic division, have been observed side by side within the same ovule (fig. 7). In another case (fig. 8) two adjacent macrospore mother cells have developed unequally; in one the homoeotypic division has been completed and cell plates formed, whereas the other has developed to the pachytene stage of the heterotypic division. In the latter cell, disintegration appears to have begun.

The functional macrospore mother cell, slightly pointed at the micropylar end, elongates until it is nearly three times as long as wide. Its granular cytoplasm becomes increasingly vacuolate but the vacuoles remain small, especially in the vicinity of the nucleus which is almost as large in diameter as the cell itself. The deeply staining nucleolus is surrounded by a light staining perinucleolar zone.

As the nucleus of the functional macrospore mother cell enters the heterotypic prophases, its resting reticulum becomes resolved into an irregular mass of leptotene threads. These threads appear to form a continuous spireme, which at first is extremely fine but later becomes thicker and contracts into a synizetic knot at one side of the nucleus (figs. 4, 5). Later the twisted and coiled threads of the spireme loosen from the knot (figs. 6, 7) and extend throughout the nuclear cavity, forming an open spireme (fig. 9). The spireme appears to be uniform in thickness throughout its length but later it shortens and thickens. Early stages in diakinesis have been observed in which several pairs of chromosomes are present, but portions of the spireme remain uncontracted. In one cell (fig. 10) nineteen pairs of chromosomes are present but several long portions of the spireme are uncontracted. The pairs of chromosomes, as seen in diakinesis, appear

short and thick, and because of their extremely small size, no constant differences in form or size could be determined.

At the equatorial plate stage (fig. 11) the axis of the spindle is parallel with the long axis of the macrospore mother cell. Occasionally a lagging of two or more chromosomes on the spindle is noted in the heterotypic anaphases.

In the macrospore mother cell shown in figure 12, the chromosomes have reached the poles and are becoming closely aggregated. Nuclear membranes inclose each daughter nucleus. At interkinesis the long spindle fibers appear to shorten and separate from the nuclei, and a cell plate is formed. By the addition of peripheral fibers, the spindle becomes barrel-shaped and the cell plate is extended entirely across the cell (fig. 13), dividing it into two daughter cells of approximately equal size. During interkinesis the two daughter cells increase somewhat in size.

Early homoeotypic prophases were not seen. The daughter chromosomes seem to move somewhat irregularly to the poles of the homoeotypic spindles. In a few instances some chromosomes precede the main group to the poles (fig. 14). In others, lagging chromosomes were observed in the late anaphases and early telophases of this division (figs. 15, 16). Lagging chromosomes were observed in both daughter cells, but they were more numerous in the chalazal cell. In several cells in both meiotic divisions it appeared that the lagging chromosomes were at some distance from the poles when nuclear membrane was beginning to form. No extra-nuclear chromosomes or small extra nuclei were observed. STEBBINS (**18, 19**) found that in the pistillate plants of several species of *Antennaria* irregularities in the meiotic divisions frequently occur. Lagging chromosomes are present in the heterotypic anaphases and extra nuclei are formed. FUKUDA (**6**) reported the presence of lagging chromosomes and extra nuclei in the microspore mother cells of potato in which the meiotic divisions occurred irregularly. He concluded that morphologically abortive pollen grains were produced by irregular and incomplete meiotic divisions.

In the Irish Cobbler the chalazal macrospore of the linear tetrad (fig. 17) always becomes the functional spore. This spore enlarges, the other three spores remaining nearly equal in size and ultimately

degenerating (figs. 18, 19). JÖNNSON (11) was the first to show that a linear tetrad of macrospores is formed in the Solanaceae. KRÜGER (12) observed a normal linear tetrad of macrospores in *S. nigrum* and in *S. tubingense*, but, contrary to the usual condition, the micropylar cell persists as the functional macrospore.

However, variations from this regular procedure have been reported in *Solanum*. NANNETTI (16) found that the development of the female gametophyte of *S. muricatum* follows that of the so-called "tulip" type. He observed no divisions which would indicate the formation of a linear tetrad of macrospores, nor did he note any traces of macrospores which may have degenerated. Somewhat later, YOUNG (21) reported that the archesporial cell in *S. tuberosum* "grows rapidly, however, and the nucleus soon becomes quite large. At this stage it may be regarded as a megaspore; further development is delayed for a time. . . . . In a few instances a row of two or three sporogenous cells was formed in the axis of the ovule, suggesting a transverse division of the original archesporial cell, though it is apparent that this is not the ordinary method of megaspore formation."

YOUNG states that, "owing to the lack of cell-division stages in the ovules in the material examined, it was not possible to determine at what stage chromosome reduction takes place, though the chromosome number was found reduced at the first nuclear division in the embryo sac." The first nuclear division in the embryo sac as shown by him (21, pl. XXVI, figs. 1, 2) resembles my figures of the heterotypic equatorial plate. The method of development of the embryo sac described by YOUNG has been interpreted by SCHNARF (17) as of the "lily?" type. The present investigation indicates that macrosporogenesis resulting in a tetrad of macrospores is the normal type of development in *Solanum tuberosum*.

These observations in *S. tuberosum* are substantiated by the condition which has been observed by KRÜGER (12) in *S. nigrum*, *S. tubingense*, and *S. proteus*, and by BHADURI (1) in *S. melongena*. In each of these species a linear tetrad of macrospores is formed. In view of the uniform method of spore formation in all these species, it is suggested that further investigations of *S. muricatum* are necessary to determine whether NANNETTI's observations of this species

are correct or whether the normal type of macrospore formation prevails here also.

### Development of macrogametophyte

The functional chalazal macrospore increases in length in part by the enlargement of vacuoles near each end of the cell. The first nuclear division occurs near the center of the cell, which has almost doubled in length. In a late anaphase of the first division of the macrospore nucleus (fig. 20), it is observed that the axis of the spindle is parallel to the long axis of the macrospore. By the time this nuclear division has been completed, the micropylar macrospores have degenerated so that only three small, lens-shaped, deeply staining bodies remain.

After the first division, the daughter nuclei, approximately equal in size, move apart and come to lie near opposite ends of the young embryo sac; between them is a large vacuolate region (fig. 21). The cytoplasm surrounding the nuclei is denser than in any other portion of the cell. At this stage the embryo sac grows rapidly both in length and in width, and becomes curved.

The chalazal and micropylar nuclei of the 2-nucleate embryo sac divide simultaneously (fig. 22). The spindles of this division are perpendicular to the long axis of the embryo sac. The resulting four nuclei (fig. 23) are respectively smaller than each of the two formed in the preceding division. The spindles persist after the formation of the four nuclei, but a cell plate has never been observed at this stage.

All four nuclei divide simultaneously, so that there are four nuclei in each end of the embryo sac. The spindles of the third division lie at either oblique or right angles to one another. In the embryo sac shown in figure 24 *B*, a portion of the spindle persisting from the second division is seen in the micropylar end of the embryo sac, extending laterally from one of the dividing daughter nuclei. One anaphase figure (fig. 24 *A*) lies so obliquely that a polar view of each end of the spindle is presented. Many young 4-nucleate embryo sacs and mature macrogametophytes were observed. The division of the four nuclei or stages showing the presence of eight nuclei preceding cell division was found, however, in only a few instances.

The four nuclei at each end of the embryo sac resulting from the

third division are connected by fibers which have persisted from both second and third division spindles. In several 8-nucleate embryo sacs, as in the one shown in figure 25, the four nuclei at the chalazal end of the sac were observed to be connected by large barrel-shaped phragmoplasts, on each of which a cell plate was in the process of formation. At the micropylar end of the embryo sac (fig. 25) there is a conspicuous phragmoplast between one pair of sister nuclei, which continues to extend laterally until it comes in contact with the wall of the cell near its tip. The spindle between the other pair of sister nuclei does not show, in this case, a cell plate. To judge from its length and position, apparently the long spindle between these two pairs of nuclei is that which persisted after the second nuclear division. An additional short spindle is seen between two adjacent nuclei.

At a later stage (fig. 26) the phragmoplasts have extended laterally to the cell wall, and a cell plate has been formed so as to cut off completely a cell at each end of the embryo sac. There is a minute groove at each end of this cell plate, apparently indicating a splitting at the margin. A few traces of spindle fibers remain. The phragmoplast with its cell plate has enlarged, curved, and extended laterally until it has reached the periphery of the embryo sac. In the micropylar end of the embryo sac, shown in figure 26 *A*, a long spindle lies parallel to the short axis of the sac between two nuclei which are not sister nuclei. The formation of the cell plate on this spindle has been delayed considerably, but it appears to extend at right angles from the vacuole to the membrane which bounds the newly formed cell just described. At the chalazal end, a membrane is formed parallel to the longitudinal axis of the embryo sac (fig. 26 *B*) between two sister nuclei and at right angles to the cell plate present on the spindle persisting from the second division. This membrane extends into the sac obliquely to meet the membrane which bounds the lateral cell already formed and thus cuts off a second antipodal cell. The cell plate on the long spindle (fig. 26 *C*) apparently will function in separating the third antipodal cell from the fourth nucleus of the chalazal group; the latter remains in the main body of the embryo sac, presumably to function as a polar nucleus.

In other views of the chalazal end of the embryo sac, the phrag-

moplasts connecting sister nuclei of the third division with their cell plates are perpendicular to each other. A cell plate is visible also on the spindle which has persisted from the second division. The three cell plates which are present delimit the three antipodals, and the fourth nucleus remains as one of the polar nuclei (fig. 27).

MOTTIER (15) found that cell division in the *Lilium* macrogametophyte results from the formation of cell plates on the spindles following the third division of the nuclei. According to MCALLISTER (14), in *Medeola virginiana* evanescent cell plates are formed between each pair of nuclei resulting from the homoeotypic division, and these cell plates may persist until after the third nuclear division. JOHANSEN (10) observed that in *Hartmannia tetraptera* the fibers of the micropylar spindles concerned in the second and final division "initiate the formation of the walls of the synergids." COOPER (4) noted that in *Lilium henryi* the cells of the egg apparatus and the antipodal cells are delimited as a result of cell plate formation across the spindles of the third division and the persistent spindles of the preceding division. In *S. tuberosum* the original wall of the embryo sac, to which the cell plates extend, partially forms the boundary of each cell of the mature 7-celled macrogametophyte. The remaining boundaries of each cell apparently result from the cell plates formed on the spindles of the second and third divisions. The cell plates which are formed on the persistent spindle of the second division are delayed in their development, and appear after the cell plates are present on the spindles of the third division.

Following cell formation, the antipodal cells enlarge somewhat and conspicuous vacuoles appear (fig. 28). Indication of their early degeneration may be noted before the fusion of the polar nuclei takes place. As a result of the enlargement of the macrogametophyte, the antipodals are pushed against the chalazal tissue of the ovule and become flattened (fig. 29). The protoplasm disintegrates, stains more deeply, and the cells remain as mere vestiges or disappear entirely by the time the fusion of the polar nuclei is completed. Antipodal cells which degenerate early have been reported for *S. tuberosum* (21), *S. melongena* (1), *S. nigrum* (12), and several other genera. In *Nicotiana tabacum* (9) and *Datura metel* (7) the antipodals enlarge

and persist for some time after development of the endosperm is initiated.

The cells of the egg apparatus are somewhat elongated. In their early stages the synergids appear to be small triangular cells pointed at the micropylar end. A nucleus lies in the dense cytoplasm near the center of each (fig. 28). By continued growth the synergids elongate, the chalazal ends become slightly broader and rounded, and a large vacuole forms in the basal portion of each cell. A nucleus remains in the dense cytoplasm immediately anterior to the large vacuole. The micropylar ends of the synergids elongate, become pointed, and extend a short distance into the micropyle. The synergids develop a distinct filiform apparatus whose striations arise in the vicinity of the dense cytoplasm anterior to the nucleus and extend to the apex of each cell (fig. 29). These striations appear as dense areas in the cytoplasm, alternating with elongated vacuoles, both of which extend parallel to the long axis of the cell. A similar condition appears in *Lycopersicon esculentum* (3).

Simultaneously with growth of the synergids, the egg increases in size until its broad basal portion extends into the gametophyte beyond the synergids (figs. 28, 29). A large vacuole develops in the apical end of the mature egg, whose nucleus remains imbedded in the denser cytoplasm of the broad basal region. The egg nucleus is somewhat larger than that of a synergid.

In the mature macrogametophyte, one polar nucleus is derived from the group of four nuclei present at each end of the embryo sac. The chalazal polar nucleus migrates to a position just posterior or lateral to the egg where it meets the micropylar polar nucleus. In some embryo sacs both polar nuclei move toward each other, meeting near the center of the sac. They remain closely appressed for a short time before fusing. Fusion of the polar nuclei occurs before fertilization. The large fusion nucleus usually occupies a position just below the egg, so that it is partially obscured in sectional view by this cell (figs. 29, 30). Fusion of the polar nuclei has been found to be completed before fertilization in *Datura laevis* (9), *D. metel* (7), *Lycopersicon esculentum* (3), and *Solanum proteus* (12). Not only this fusion but also the first division of the primary endosperm cell as well occurs before fertilization in *Petunia*, according to FERGUSON (5).

## DEGENERATION

Macroscopic evidences of degeneration in *Solanum tuberosum* may be observed in buds of all sizes as well as in open blossoms. The buds which degenerate cease to grow, become yellowed, and separate from the plant at a definite abscission region in the pedicel. If anthesis occurs, many of the flowers wither very soon. In some flowers which open, the anthers appear to be shriveled and twisted. Fruits were never observed on the plants from which the material was collected.

In many buds the microsporogenous tissue has broken down completely, leaving only a densely staining mass of material in the locules of the anthers. In some of the ovules of these buds, the macrospore mother cell appears to be arrested in its development, as indicated by its unelongated condition and its deeply staining protoplasm. In some older buds the anthers appear normal in shape and size. Even in these, however, many of the microspores are shrunken and disintegrated. In these buds many of the ovules contain embryo sacs which appear to be normal; evidently not all the ovules of an individual pistil may be involved in disintegration. Pollen tubes were never observed in the styles of pistils which had been dissected out of open flowers. YOUNG (21) found that the changes which indicate degeneration in the ovules usually accompany those irregular meiotic divisions which occur frequently in the pollen mother cells; disintegration within the ovules is less frequent than that which occurs within the anther.

Degeneration may occur within the ovule of *S. tuberosum* at any stage, but it is rarely observed in very young stages. When a multiple archesporium is present, with occasional exceptions all but one of the archesporial cells disintegrate or fail to continue their development, a single cell then remaining to function as a macrospore mother cell. In some ovules meiotic divisions occur, but all four macrospores disintegrate instead of the usual three micropylar spores. In a few ovules the micropylar daughter cell was observed to degenerate completely after the heterotypic division, but the nucleus of the chalazal daughter cell completed the homoeotypic division. Cell division followed and two macrospores were formed.

Disintegration occurring immediately after the meiotic divisions, and resulting in complete disorganization of the macrospores, may be the result of irregularities in chromosome distribution. Lagging chromosomes on both heterotypic and homoeotypic spindles were observed in some ovules (figs. 15, 16). Whether or not such chromosomes are included in the daughter nuclei or remain in the cytoplasm to form supernumerary nuclei could not be determined, since many of the cells degenerate to such an extent that all internal structure is obliterated.

When degeneration takes place in the young macrogametophyte, the embryo sac shrinks away from the inclosing integument, stains heavily, and presents a shriveled disorganized appearance. The shrinkage begins usually at the micropylar end of the sac. Deep-staining masses are present within some ovules which may be 2- or 4-nucleate embryo sacs that have been almost completely disorganized. In other instances the micropylar nuclei of the young macrogametophyte were observed to have disintegrated completely, while those at the chalazal end appeared nearly normal. The cytoplasm in such cases becomes fibrillar in appearance and stains more heavily than in the normal macrogametophyte. This suggests that degeneration may not be a sudden occurrence involving the entire gametophyte at the same time, but rather may be progressive (fig. 31). In some cases the integumentary tissue inclosing a degenerating embryo sac is normal in appearance; in other ovules the nutritive cells of the integument degenerate completely with the embryo sac they inclose.

Degeneration may follow the third nuclear division in the embryo sac (fig. 32). The peculiar shape of the embryo sac here shown is due to its shrinkage within the ovule as well as to the plane in which the section was cut. A dense cytoplasmic zone in which are distinguishable many small granules is present in the center of the sac and is surrounded by large vacuoles. Eight nucleoli and a delicately staining zone inclosing each are the only visible traces which remain of the nuclei of the macrogametophyte. Apparently degeneration had set in before the initiation of cell division. BRADBURY figured a similar degree of degeneration in the egg nucleus and the fusion nucleus in

unpollinated blossoms of *Prunus cerasus* (**2**, pl. 53, fig. 17). The nucleoli are the only distinguishable remains of the egg nucleus and the fusion nucleus.

In some embryo sacs the micropylar ends of the cells of the egg apparatus stain very heavily and all structural details are obliterated. Only the nucleoli can be differentiated within the nuclei. Occasionally nuclear material is visible about the nucleolus of the egg, although scarcely distinguishable from the surrounding cytoplasm.

In one mature macrogametophyte, which appears to be degenerating, the egg occupies a rather unusual position (fig. 30). It appears to lie at an angle of about 160° from the normal. The apex of the cell is directed toward the side of the primary endosperm cell, and the broad curved end lies above the synergids. The nucleus appears densely granular and has a greater affinity than usual for stains. The primary endosperm nucleus may be seen below the egg. The synergids lack a distinct filiform apparatus, but the cytoplasm at their micropylar ends is somewhat reticulate and stains heavily. The reticulate nature of the cytoplasm of the synergids, the altered position of the egg, the granular appearance and deeply staining quality of the egg nucleus, and the increased vacuolation of the embryo sac may be considered evidences of degeneration. The breaking down of the cells, however, may be a result of the failure of fertilization.

These irregularities which occur during macrosporogenesis and the development and maturation of the macrogametophyte may partially account for the failure of seed and fruit development in the potato. There seems to be some correlation, however, between the irregularities occurring during microsporogenesis and the development of the pollen grains on the one hand, and those taking place during macrosporogenesis and macrogametophyte development in the ovules of the same flower on the other hand. Further investigation should determine if possible the relationships of these irregularities to the sterility which is so common in the potato.

## Summary

1. A single archesporial cell, hypodermal in origin, may function as a macrospore mother cell. Frequently, however, a multiple arche-

sporium is differentiated from hypodermal and subhypodermal cells of the nucellus. In such case each archesporial cell may function as a macrospore mother cell, but usually only one of them so functions.

2. A linear row of four macrospores is formed. The chalazal macrospore develops into an embryo sac; the other three spores disintegrate.

3. The micropylar cells of the nucellus break down and the developing embryo sac comes into contact with the inner epidermis of the integument, which functions as a nutritive layer.

4. In the 4-nucleate embryo sac the spindle persists between each pair of sister nuclei, but no cell plate has been observed at this stage.

5. A typical 8-nucleate embryo sac is formed, at each end of which the four nuclei are connected with one another by fibers from the spindle of the third division as well as by persistent spindle fibers from the second division.

6. Cell division occurs by the formation of cell plates across the spindles of the second and third divisions in the embryo sac.

7. Changes involving degeneration within the ovule may occur at any time during macrosporogenesis and during development of the macrogametophyte.

8. Macrosporogenesis may be accompanied by irregularities in chromosome distribution. Chromosomes, lagging on the spindle, were observed in several instances in both heterotypic and homoeotypic divisions.

9. Degeneration in the microsporogenous tissue may be associated with corresponding changes in the ovules. If degeneration occurs late in the anthers, only a small proportion of the developing embryo sacs give any indication of breaking down.

10. Degeneration of mature macrogametophytes occurs frequently.

11. Pollen tubes were not observed in the style of any pistil examined.

12. Degeneration within the ovules may account to a certain degree for the failure of seed formation in the potato.

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## EXPLANATION OF PLATES IX, X

All drawings were made with an Abbé camera lucida at table level. Bausch and Lomb compensating oculars, a Leitz periplan 20× ocular, a Bausch and Lomb 4 mm. objective, and an F. Koristka oil immersion 1/12", 1 mm. omog. AP. N. 1.36 objective were used, except for figures 26 and 27, which were made with Spencer compensating oculars and a Leitz oil-immersion N.A. 1.32 objective.

### PLATE IX

FIG. 1.—Very young ovule containing two archesporial cells. ×425.

FIG. 2.—Young ovule containing a single archesporial cell. ×425.

FIG. 3.—Young ovule containing four archesporial cells. ×425.

FIG. 4.—Young ovule containing two macrospore mother cells, nucleus of each being in an early meiotic prophase. ×425.

FIG. 5.—Synizesis. ×1050.

FIG. 6.—Nucleus recovering from synizesis. ×1050.

FIG. 7.—Two adjacent macrospore mother cells with nuclei recovering from synizesis. ×1050.

FIG. 8.—Macrospore mother cell with nucleus in open spireme adjacent to a linear dyad in a late telophase of homoeotypic division. ×800.

FIG. 9.—Open spireme. ×1750.

FIG. 10.—Early diakinesis with 19 pairs of chromosomes and portions of spiremes present. ×1750.

FIG. 11.—Macrospore mother cell with heterotypic equatorial plate. ×1050.

FIG. 12.—Macrospore mother cell within ovule; heterotypic telophase showing cell plate formation. ×1050.

FIG. 13.—Interkinesis; spindle showing cell plate formation. ×425.

FIG. 14.—Daughter cells during homoeotypic division; early anaphase. ×1050.

FIG. 15.—Daughter cells during homoeotypic division; anaphase stage with chromosome lagging on spindle of each cell; cell plate forming in chalazal cell. ×1050.

FIG. 16.—Daughter cells during homoeotypic division; early telophase stage with chromosomes lagging on spindle of each cell. ×1050.

PLATE X

FIG. 17.—Daughter cells formed after homoeotypic division is completed; cell plates across each daughter cell. ×1050.

FIG. 18.—Typical linear tetrad of macrospores with three micropylar spores beginning to disintegrate. ×1050.

FIG. 19.—Typical linear tetrad of macrospores, chalazal spore enlarging and three micropylar spores disintegrating. ×1050.

FIG. 20.—Division of macrospore nucleus. ×1050.

FIG. 21.—Two-nucleate embryo sac; nuclei in early prophase stage of division. ×800.

FIG. 22.—Embryo sac; division figures present, leading to 4-nucleate stage. ×1050.

FIG. 23.—Four-nucleate embryo sac; persistent spindle between sister nuclei. ×800.

FIG. 24.—Division stages leading to 8-nucleate embryo sac: *a*, polar view of late anaphase of dividing nucleus; *b*, portion of spindle persistent from second division. Composite drawing from two serial sections of same embryo sac. ×800.

FIG. 25.—Division stages leading to 8-nucleate embryo sac; phragmoplasts between sister nuclei at chalazal end and one pair of sister nuclei at micropylar end of embryo sac; spindles present between remaining nuclei in micropylar region. Composite drawing from two serial sections of embryo sac. ×800.

FIG. 26.—Four nuclei in each end of embryo sac; antipodals and egg apparatus in process of formation by means of cell plates present on spindles of third division and those persisting from second division. Composite drawing from two serial sections of embryo sac. ×725.

FIG. 27.—Chalazal end of embryo sac: *a*, cell plate on spindle between sister nuclei, polar nucleus and an antipodal nucleus; *b*, cell plate on spindle persisting from previous division. ×725.

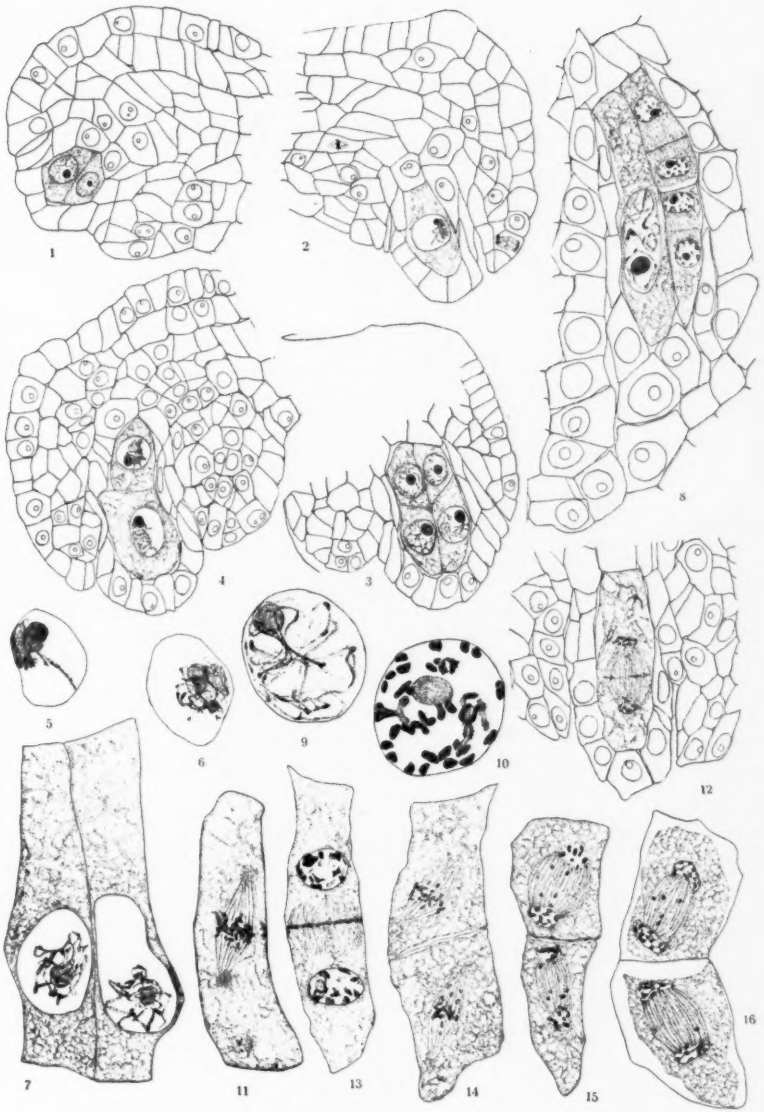
FIG. 28.—Immature 7-celled macrogametophyte. Composite drawing from two serial sections of embryo sac. ×800.

FIG. 29.—Mature macrogametophyte, antipodals disintegrating. ×800.

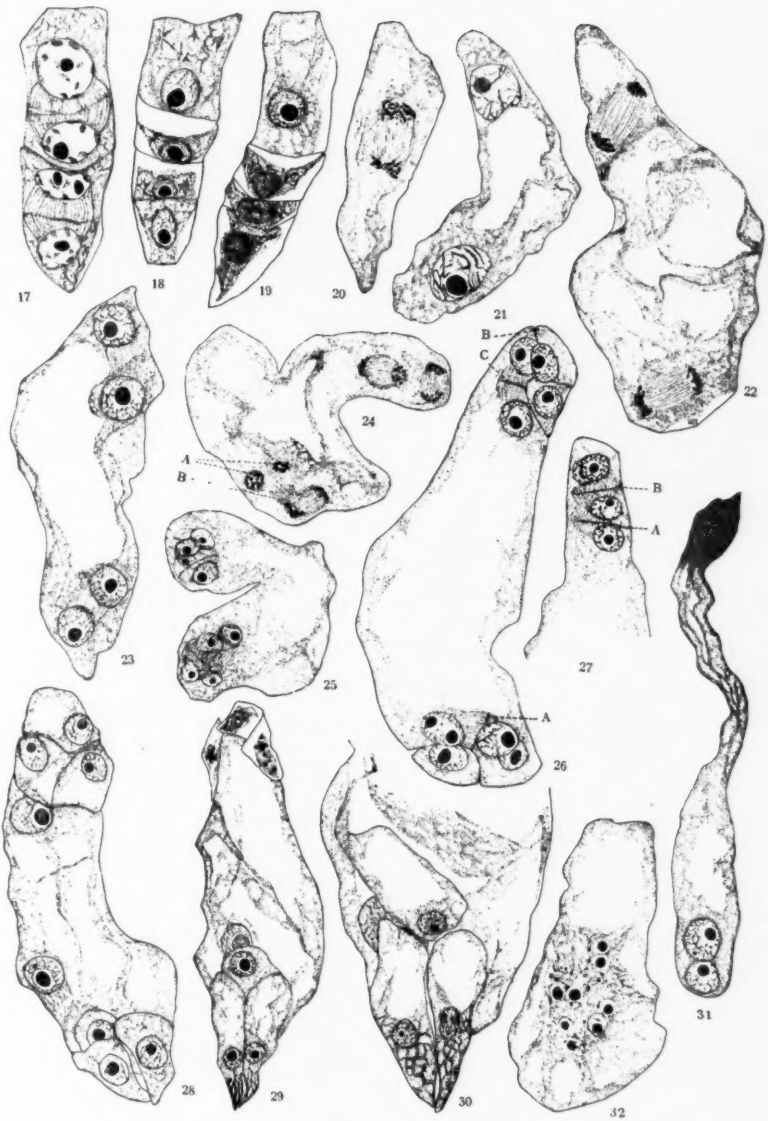
FIG. 30.—Apical portion of embryo sac showing micropylar apparatus with fusion nucleus; position of egg altered. ×800.

FIG. 31.—Disintegration of 4-nucleate embryo sac; micropylar end of sac involved. ×800.

FIG. 32.—Degeneration of 8-nucleate embryo sac. ×800.




REES-LEONARD on SOLANUM




REES-LEONARD on SOLANUM

# THE MONIMIACEAE AND A NEW LAURELIA

EDWARD W. BERRY

(WITH TWO FIGURES)

The family Monimiaceae does not reach the North Temperate zone, consequently it is unfamiliar to students of fossil floras who have largely lived in that zone. It is a family of great interest to students of plant distribution. Small in size, it contains between 25 and 30 genera and between 175 and 200 species. Systematists have usually recognized a relationship with the Lauraceae and now both families are included in the order Laurales. With the exception of *Laurelia*, no genus is common to both the Old and the New Worlds. Three genera are confined to New Caledonia and only *Tambourissa* of the Old World genera is found in more than one continental area. Nearly half of the known genera are monotypic in the existing flora. But two genera have more than a few species, and both of these, *Mollinedia* and *Siparuna*, with more than 100 species between them, are confined to America. The pertinent facts of the modern distribution are summarized on the accompanying map (fig. 1), to which the reader is referred in lieu of a lengthy discussion.

Only the following six genera have been recognized in the fossil state: *Hedycaria*, *Peumus*, *Mollinedia*, *Monimia*, *Laurelia*, and *Siparuna*, and several of these are of somewhat uncertain status. In addition SAPORTA described three species from the basal Eocene of France for which he proposed the extinct genus *Monimiopsis*.

Four species of *Hedycaria* have been recorded from the Oligocene and Miocene of Europe and a fifth from the Tertiary of Australia. The genus *Mollinedia* has been recorded from the European Miocene and from the Tertiary of Seymour Island. *Monimia* has been recorded from the Oligocene of southern Europe and from Australia. *Siparuna* has been recognized in a warm climate Eocene flora from Oregon and it seems likely that it may be represented in the Eocene floras of southeastern North America.

Six fossil species have been referred to *Laurelia*, and these are

referred to in connection with the following description of a new species from northern Patagonia. The latter comes from a locality on the Rio Pichileufu in northern Rio Negro Territory, about 30 miles east of Lago Nahuel Huapi (latitude 41° 10′ south, longitude 70° 52′ west).

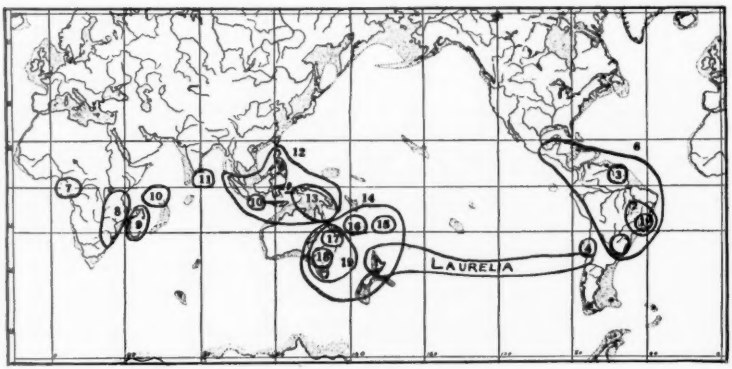



FIG. 1.—Map showing geographical distribution of genera of Monimiaceae: 1, *Macrotorus;* 2, *Macropeplus;* 3, *Conuleum;* 4, *Peumus;* 5, *Hennecartia;* 6, *Siparuna* and *Mollinedia;* 7, *Glossocalyx;* 8, *Xymalos;* 9, *Ephippiandra;* 10, *Monimia* and *Tambourissa* in Mascarenes, *Tambourissa* in Java; 11, *Hortonia;* 12, *Matthaea* and *Kibara;* 13, *Levieria* and *Palmeria;* 14, *Hedycarya;* 15, *Trimenia;* 16, *Amborella, Carnegiea,* and *Nemuaron;* 17, *Piptocalyx* and *Daphnandra;* 18, *Doryphora;* 19, *Antherosperma.*

**Laurelia guinazui** Berry sp. nov.

Leaves of variable size, broadly lanceolate to oval in outline with cuneate base and pointed apex, usually widest near the middle and narrowed about equally proximad and distad. Margins entire at base, elsewhere with prominent serrate teeth, which are conspicuously glandular tipped. Texture subcoriaceous. Length ranging from 5 to 12 cm., normally 7.5 to 11 cm. Maximum width ranging from 2.4 to 6 cm., normally 3 to 3.5 cm. Petiole relatively very stout, usually curved, slightly over 1 cm. in length. Mid-vein stout, not especially prominent. Secondaries six or seven ascending, regularly curved camptodrome pairs, giving off prominent tertiary branches to the marginal teeth. Tertiaries forming an open mesh. Areolation fine, polygonal, isodiametric (fig. 2).

These leaves have the general form and toothed margin similar to that found in a variety of unrelated genera of Flacourtiaceae, Sapindaceae, Proteaceae, and various other families but differ in

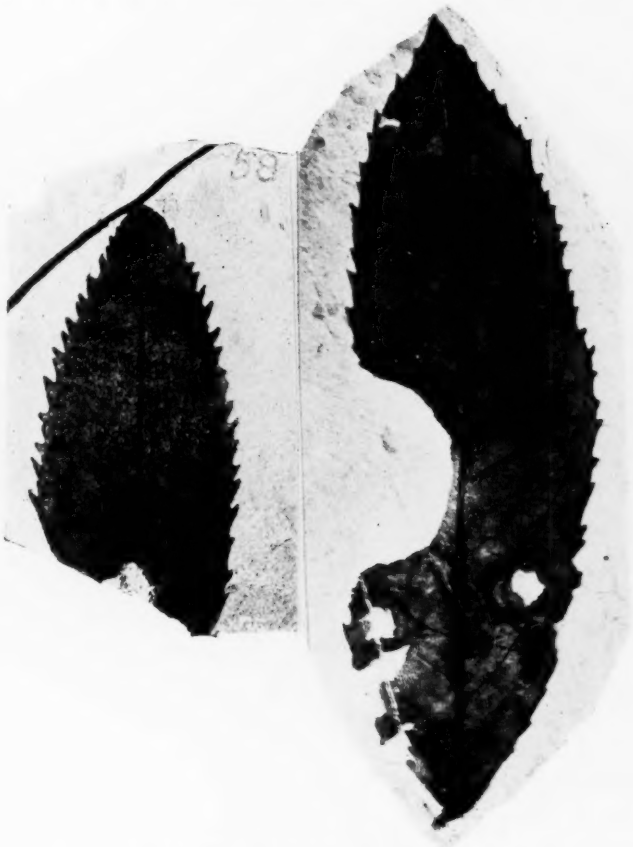


FIG. 2.—Limits of variation in *Laurelia guinazui* Berry, sp. nov.

having glandular marginal teeth. It is this last feature added to the general similarity of the other features to the leaves of the existing species of *Laurelia* that confirms the identification.

The genus is a small one in the existing flora found in Chile and

the northern part of New Zealand. The two Chilean species, *L. sempervirens* and *L. serrata*, occur near the western Andean border of the territories of Rio Negro and Chubut, not far from the fossil locality, in the former approaching to within perhaps 30 or 35 miles of the latter.

The geological record is very incomplete and inconclusive because of the difficulty of recognizing the leaves, but the present day distribution of the surviving species is conclusive enough evidence that an extended geological history awaits discovery. LESQUEREUX many years ago described a species from the Dakota sandstone of Kansas, but this can hardly be accepted as conclusive evidence of the existence of the genus in the mid-Cretaceous of North America. Similarly ENGELHARDT described a species from the Upper Eocene of Germany which is equally doubtful. A European Miocene species described from Croatia by UNGER is probably a member of the family Ternstroemiaceae. ETTINGSHAUSEN described a species from the Lower Miocene of Kutschlin, Bohemia, which looks very like *Laurelia*, but about which no certainty can be expressed. DUSÉN[1] has described a species, considered to be of Oligocene age, from Seymour Island on the border of Antarctica, and a second Argentine species was described[2] a few years ago from Santa Cruz territory.

The present species differs from the last of these, *Laurelia amarillana* Berry, in its average size being somewhat larger, in its slightly more oblong shape, in its more curved and wider initial divergence of the secondaries, in its more regular teeth, and in its more conspicuous glands. In all of these features it departs in the same way from the existing *Laurelia* of South America, *Laurelia amarillana* being practically identical with the latter.

Both of these fossil South American species as well as that from Seymour Island are much more like the existing South American species than they are like the existing New Zealand species. The last has leaves which are smaller and rounder and with more typically crenate marginal teeth.

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[1] DUSÉN, P., Schwed. Südpolar-Exped. **3**: p. 4, pl. I, fig. 5. 1908.

[2] BERRY, E. W., U.S. Natl. Mus. Proc. **73**: p. 21, pl. V, fig. 3. 1928.

# THE LIGHT LINE IN MELILOTUS ALBA

DOUGLAS H. HAMLY

(WITH FOUR FIGURES)

## Introduction

In a previous article on the seeds of *Melilotus alba*, the writer (3) disagreed with the opinions of former investigators concerning the nature of the "light line" which crosses the Malpighian cells just within the caps. At that time it was not possible definitely to demonstrate the cause of its appearance; hence the present paper gives the results of further study of these light lines.

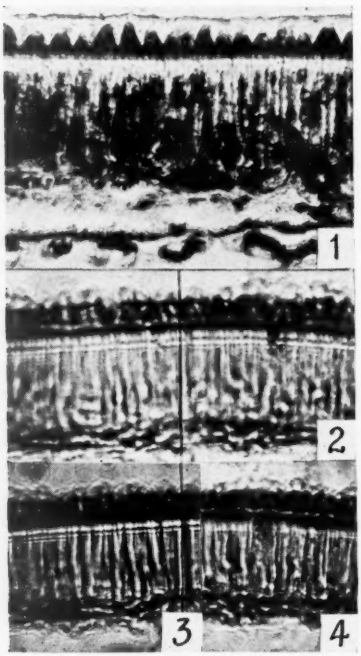
## Investigation

Under ordinary conditions of observation with diffused light, the light line in *Melilotus* appears as a single, rather broad band. It crosses the cells at the interface between the suberin caps and the cellulose bases as described in the paper just cited. This interface is easily seen under the usual conditions of observation. Irregularity may occur, however, as may be observed in a series of photographs made with ultraviolet light by Dr. A. KÖHLER of Jena. Since suberin is much more opaque than cellulose to this light, the caps appear very dark (fig. 1). In the original photographs the configuration of the interface, the finger-like processes of cellulose around the lumen, and the fringed margin of the suberin caps are clearly visible.

With carefully adjusted non-polarized illumination the light band may be resolved into two separate lines (fig. 2), one line occurring within the cellulose and the other within the suberin. The independence of these lines is demonstrated as the objective of the microscope is raised above the plane of best focus.

Thus analyzed, the broad and somewhat hazy light line is seen as two lines, each behaving as a BECKE (1) line such as is found at the surfaces of adjoining transparent substances which differ in refractive index. The BECKE line always moves toward the substance of higher index as the tube of the microscope is raised.

The evidence that the light line is in reality two associated BECKE lines is confirmed by examining the material in polarized light (figs. 3, 4). Figure 3 is a photograph made with light polarized in a plane parallel to the axis of the Malpighian cell. Under these conditions the faint line within the suberin caps is absent, and only the brighter line on the cellulose side remains. The light in figure 4 is polarized in a plane at right angles to the axis of the Malpighian cell. In this case the line is visible in the suberin while that in the cellulose has disappeared. For figures 2, 3, and 4, the lighting was arranged according to the method of KÖHLER (4). To emphasize the separation of the lines the photographs were made from sections placed slightly below the plane of sharp focus.




FIGS. 1–4.—Fig. 1, photograph of Malpighian cells taken by ultraviolet light with Cd 0.257 $\mu$ line, N.A. 0.15. ×508 (KÖHLER). Figs. 2–4, photographs of same 15 $\mu$ section from the same plane slightly above plane of best focus. (KÖHLER illumination with ribbon filament lamp as the source. Wratten M filters C and H used in combination.) ×455: fig. 2, in non-polarized light; fig. 3, in light polarized parallel to axes of Malpighian cells; fig. 4, in light polarized perpendicular to axes of Malpighian cells.

The immersion methods of CHAMOT and MASON (2) were used to determine the refractive indices of the Malpighian cell constituents. The data for the refractive indices given in table I differ slightly from those reported for pure cutin and pure cellulose.

With light vibrating parallel to the axis of the Malpighian cells the index of the cellulose base is greater than that of the suberin cap by 0.031, a difference great enough to produce a bright BECKE line which moves toward the cellulose as the tube of the microscope

is raised. When the plane of polarization is perpendicular to the axis of the cell the index of the suberin exceeds that of the cellulose by 0.019. This produces a faint BECKE line on the suberin side of the surface of contact. It is thus definitely indicated that the conditions produced by the juxtaposition of the cellulose and the suberin are such as must give rise to the phenomenon known as the light line.

TABLE I

REFRACTIVE INDICES OF MALPIGHIAN CELL CONSTITUENTS

| MATERIAL | POLARIZATION OF LIGHT | |
|---|---|---|
| | PARALLEL TO AXES OF MALPIGHIAN CELLS | PERPENDICULAR TO AXES OF MALPIGHIAN CELLS |
| Suberin caps | 1.554±0.0005 | 1.556±0.0005 |
| Cellulose walls below the caps | 1.585±0.003 ($n_\alpha$) | 1.537±0.003 ($n_\beta$) |
| Difference in refractive index | 0.031 | 0.019 |

The writer gratefully acknowledges the assistance of Dr. A. KÖHLER, and the advice and criticism of Professor H. B. SIFTON, of the Department of Botany and Professor A. L. PARSONS, of the Department of Mineralogy and Petrography, of the University of Toronto.

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# SEED AND SEEDLING OF ACORUS CALAMUS

MURRAY F. BUELL

(WITH THREE FIGURES)

*Acorus calamus* L. grows in eastern and tropical Asia, in Europe, and in eastern North America. In Europe, where it has been introduced and naturalized within historic times, it appears to be uniformly sterile. This is also true of much of the material growing in the older-settled parts of the United States, material which was probably introduced from Europe. In the northern interior, however, where it has every appearance of being native, it fruits abundantly.

It would seem that a study of the seed and seedling of this plant has been neglected in this country, and European observations apparently have been perforce limited to the earliest stages of the ovules. Both MÜCKE (4) and JÜSSEN (3), investigating the causes of the sterility of the European plant, found early degeneration of the embryo sac and very few normal pollen grains. MÜCKE also made some observations on ripe seeds of *Acorus calamus* obtained from Asia, but apparently his supply of these was very limited. He made rather full observations on the development of the seeds of *A. gramineus* Soland, a second Asiatic species. The following observations are submitted at this time; more extended studies on the embryogeny are under way.

## Observations

### FRUIT AND SEED

The fruit is a several-seeded (1–9, most frequently 5–7), three-locular, dry berry. It is 4–5 mm. long, roughly wedge-shaped, narrowing toward the base, and somewhat 4-angled as a result of crowding on the spadix. The scarious pericarp is lustrous, light brown to straw-colored, and marked with longitudinal dark brown streaks. The septa are thin and delicate. The seeds are borne in a cluster on an axile placenta near the summit of each locule. Although the ma-

ture locules are practically filled with the closely packed, crowded seeds, they contain in the interstices a clear, transparent mucilage, hard and brittle in the mature dry fruit but rapidly absorbing water and swelling into a soft gelatinous mass when dampened. Each seed is invested by a somewhat denser sheath of the mucilage. This mucilage is derived earlier in the development from glandular hairs at the base of the ovules. As a rule all the ovaries on the spadix develop seeds, although occasionally there is some abortion.

MÜCKE's (4) brief description of the fruit of Asiatic *Acorus calamus* is essentially in agreement with this, except that he found fewer

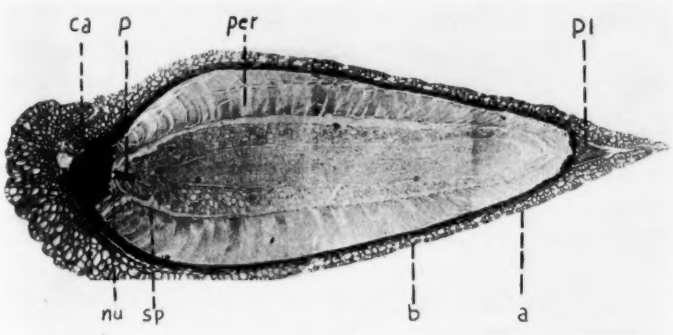


FIG. 1.—Longitudinal section of seed of *Acorus calamus*. ×31

seeds (2–5) in each fruit. EATON (2) likewise described *Acorus* fruit as fewer seeded. Both BRITTON (1) and SMALL (5) describe *Acorus* fruit as "2–3-celled," but in the present material it is regularly 3-celled; empty locules of semi-abortive fruits may readily be overlooked in the dry condition.

The orthotropous seeds are 3–4 mm. long, narrowly ovoid, and somewhat angled as a result of crowding. The chalaza tapers into the short funiculus which may be either straight or curved or nearly absent, depending upon the position of the individual seed in the locule. The outer surface is light brown, speckled with dark brown indentations. Under the microscope it glistens owing to the air filled parenchyma cells that make up the tissue of the testa. This is 3–4 cells thick, soft, spongy, and easily scraped away. When cut

or bruised it is strongly aromatic. At the micropyle it extends out beyond the nucellus in finger-like lobes.

The tegmen, derived from the inner integument, is thinner and is composed of two layers of cells. These are narrow, elongated, with pointed ends. They fit together tightly and each cell is reinforced with spiral thickenings. The result is a firm, tough, dark brown jacket. At the chalaza this tissue is interrupted by a weak cap of dead cells (fig. 1 *ca*). These are small, parenchymatous, and compactly arranged. In sections stained with safranin or haematoxylin they take an intense stain. In the micropylar region the cells of the inner integument are elongated, coiled about one another, and form a plug in the lower part of the micropyle (fig. 1 *pl*). This inner seed coat everywhere fits tightly against the perisperm. Even at the apex, where the surface of the latter is runcinate, its irregularities are filled in with a firm "cement" formed from disintegrated cells of the tegmen.

The inner cells of the nucellus become entirely disintegrated except for a few remnants which may be seen between the perisperm and the endosperm, while its outer layer persists in the ripe seed as a tough, callous, transparent perisperm one-fifth to one-quarter the thickness of the entire seed (fig. 1 *per*). It is a single layer of cells[1] forming a thick, continuous jacket about the endosperm, except at the base where it is interrupted by the cap of degenerate cells already mentioned. Its cells have the form of truncated wedges radially arranged about the embryo sac. Especially toward the chalazal end of the seed they are somewhat curved, with the concave side toward the chalaza. The walls between these cells are very thin. The cell contents are glassy-clear and homogeneous, no structure whatsoever being observable in fresh material even under the high power of the microscope.[2] MÜCKE (4), working with the corresponding tissue in *A. gramineus*, found it to be protein in composition, and states that it is absorbed at a very early stage of germination. In *A. cala-*

[1] The appearance of more than one layer seen on the upper side of figure 1 is due to the slight obliquity of the plane of the section.

[2] The granular appearance of the cell contents seen in figure 1 results from their having been treated with hydrofluoric acid. Without this procedure it is impossible to obtain microtome sections of the perisperm.

*mus* this process seems to be somewhat delayed, but during germination the perisperm soon softens, and in the later stages it disappears entirely even while the tip of the cotyledon is still surrounded by a layer of unabsorbed endosperm.

The soft endosperm is made up of thin walled parenchyma cells filled with abundant oil and protein food material. It surrounds the embryo completely. A thin layer of crushed cells belonging to the inner part of the nucellus separates it from the perisperm, and a few less crushed cells of this tissue remain about the base of the embryo sac (fig. 1 *nu*). The endosperm is homogeneous throughout except that it is a little more densely filled with stored food in the basal region. Not far from the basal end there is evident in some cases the remnant of a transverse septum which seems to indicate an early division of the embryo sac into two parts (fig. 1 *sp*).

Conspicuous in the longitudinal section of the seed is a structure similar to that which WESTERMAIER (**6**) has described (in the genus *Aconitum*) as a pedestal (Postament). This appears in the ripe seed as a hard knob of degenerate cells which stands out sharply in stained sections (fig. 1 *p*). It is the remains of a non-vascular conducting strand, which earlier served to supply the embryo sac, together with some hardened remnants of adjacent cells. The downward enlargement of the embryo sac takes place at the expense of the abundant nucellar tissue that originally surrounds this strand, but the process of disintegration has but little effect on the more resistant conducting strand itself.

The cylindrical embryo lies in the axis of the endosperm. It is about 1.5 mm. long. To the base there is still attached a small suspensor (fig. 2 *A*, *s*). The base of the cotyledon sheathes the plumule (fig. 2 *B*) while its terete limb extends far beyond, forming about three-quarters of the length of the entire embryo.

## SEEDLING

Seeds collected in September and planted in the University greenhouse germinated readily the following spring. Freezing temperatures during the winter tended to shorten the rest period. Those seeds which have undergone the normal rest period or have been subjected to freezing temperatures show signs of germinating within about

10 days. The radicle is forced through the micropyle as the cotyledon begins to elongate. Almost immediately the radicle turns downward so that the rapidly developing primary root is directly established in the soil (fig. 3 *A*, *B*). Simultaneously with commencement

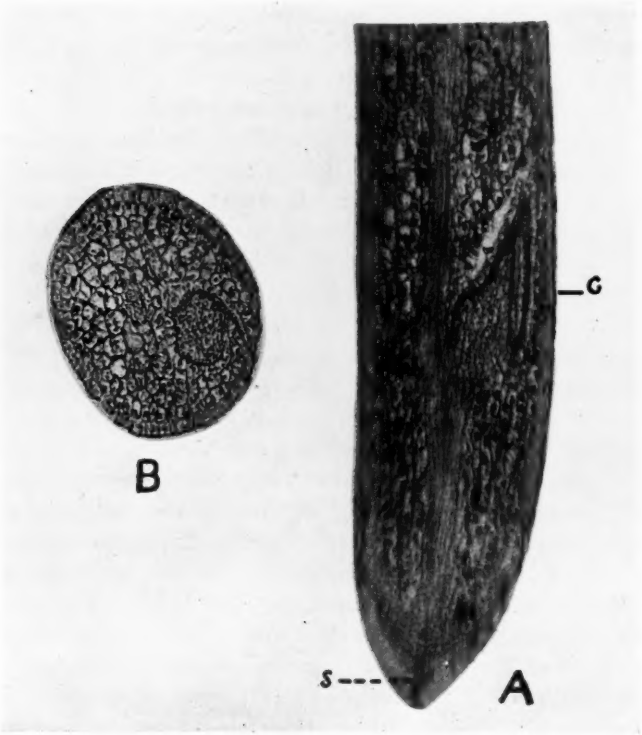


FIG. 2.—*A*, longitudinal section of embryo between *a* and *b* of fig. 1; *B*, cross section of embryo at *c* in adjacent figure. ×150.

of root growth the hypocotyl begins to enlarge slightly (fig. 3 *A*, *sw*), and at this swelling appears a band of hairs, which doubtless serve as auxiliary organs to absorb water and minerals while the primary root is yet in its embryonic stages (fig. 3 *B*, *C*, *D*, *h*). These primary absorbing organs, together with the immediate production of chloro-

phyll in the emerging embryo, lead to the early independence of the plant.

While the primary root develops rapidly, it remains small and is soon replaced by numerous adventitious roots. The cotyledon con-

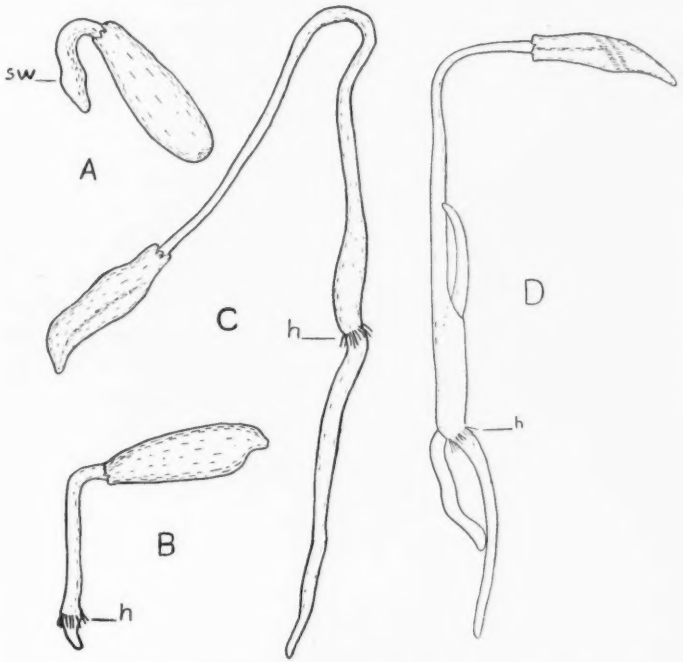


FIG. 3.—Stages in development of the seedling. ×4.2

tinues elongating, lifting the remains of the seed into the air, while its tip remains in the endosperm, absorbing the last of the stored food. At maturity of the seedling, that is, as the first plumular leaf is about to emerge, the cotyledon attains a length of about 20 mm., its sheathing base forming one-fifth to one-quarter of its whole length. There is no ligule on the cotyledon as there commonly is in monocotyledonous seedlings. The first plumular leaf appears about simultaneously with the first adventitious root (fig. 3 *D*).

In the dormant embryo the vascular system is indicated by a pro-

cambial strand which can be traced from the apex downward along the axis of the cylindrical cotyledon and into the hypocotyl region (fig. 2 *A*). Slightly above the base of the plumule it becomes more distinct. In the hypocotyl it is best developed on the side directly below the median plane of the cotyledon and somewhat less sharply differentiated on the side that lies below the plumule. The plumule itself is as yet completely undifferentiated meristematic tissue. The latter, however, develops rapidly during germination; and about simultaneously with the first conspicuous indications of conducting tissue in the rudimentary primary root, the midrib of the first plumular leaf becomes evident and at the same time there appears the first differentiated xylem in the base of the cotyledon.

In the mature seedling the vascular tissues of the cotyledon and primary root are well differentiated, and the three main traces of the first plumular leaf and a single stem bundle are readily discernible. In the limb of the cotyledon there is a single vascular strand with collateral xylem and phloem. As this passes downward through the brief transition region the exarch condition is attained and the root structure rapidly appears. The root is diarch, one pole developing directly beneath the cotyledonary strand and the other directly in line with the midrib of the first plumular leaf. The endodermis, present in the root and the rhizome of the plant, is already differentiated in the seedling at the first node.

## Summary

1. *Acorus calamus* L., which fails to produce seed in Europe, fruits freely in Minnesota, where it is probably native.

2. The fruit is a 3-celled, gelatinous, dry berry containing usually 5–7 orthotropous, ovoid, somewhat angled seeds pendent from an axillary placenta. The cylindrical embryo lies in the axis of the endosperm, which is surrounded by a thick callous perisperm. The seed coat is made up of a thin tough tegmen surrounded by a thicker spongy testa.

3. As the seed germinates the tip of the cotyledon remains in the seed as an haustorial organ and lifts it into the air as growth continues. The seedling immediately becomes green and develops absorbing hairs so that it is early independent. The single vascular

bundle of the cotyledon passes directly down into one pole of the diarch root while the midrib of the first plumular leaf is directly continuous with the other pole.

I wish to thank Dr. C. O. ROSENDAHL and Dr. F. K. BUTTERS who suggested the problem and gave valuable assistance during the course of the work.

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# CURRENT LITERATURE

### Genetics of garden plants

According to the preface, the aim of this book[1] is twofold: "First, to give an introduction to the essential principles of genetics and cytology; and secondly, to give an account of recent results in relation to horticulture." The first objective as it relates to genetics seems to be doubtfully attained, while the second is admirably handled.

The general principles of genetics are condensed into one short chapter of 12 pages. Even for the lay horticulturist, this brevity means unbalance and serious omission. Nowhere is the theory of quantitative inheritance discussed; the principles of heterosis are granted one short sentence; and most surprisingly, the whole question of inbreeding is totally ignored. Obviously no one can do justice to a field like genetics in so brief a treatment, but it may serve to spur the reader to further study.

Cytological phases are clearly and concisely developed; particularly the chapter on the cytology and genetics of polyploids is very well reviewed.

There is a noticeable tendency to minimize and overlook much of the better continental European and American research on genetics. For example, the treatment of *Dahlia* is exhaustive while that on *Antirrhinum* is almost neglected. The German, Russian, and American work on *Cucurbita*, *Lycopersicum*, *Phaseolus*, and many other garden crops is either ignored or underemphasized. The bibliography is correspondingly incomplete and unbalanced. The history and genetics of the sweet pea, the garden stock, *Primula*, the garden pea, and the potato are exceptionally well treated.

A major share of the book naturally deals with the special interests of the authors. Here the discussions on fruit breeding, genetics, and cytology are extremely well presented, as is the recent work on sterility and incompatibility. The advanced student of horticulture will certainly need this book for his field of research, and will find the material clearly and convincingly written. In fact, did the title read "Recent advances in cytology and breeding of garden plants" instead of its actual, very broad title, many of the preceding criticisms would be unnecessary. In general the format is excellent and the illustrations original and instructive.—E. W. LINDSTROM.

### Physico-chemical properties of plant saps

The untimely death of Dr. J. ARTHUR HARRIS left a great mass of uncompiled and unpublished data on the physico-chemical properties of plant saps which he

[1] CRANE, M. B., and LAWRENCE, W. J. C., The genetics of garden plants. 8vo. pp. xvi+236. figs. 53. Macmillan, London. 1934.

collected during many seasons' field work in both the eastern and western United States, the Hawaiian Islands, and Jamaica. Although very regrettable that Dr. HARRIS could not have been spared to complete this work himself, it is extremely fortunate that these data, consisting of approximately 12,000 series of determinations of freezing point depression, specific electrical conductivity, chloride and sulphate content in grams per liter of sap, and occasional determinations of hydrogen ion concentration, have been made available in book form for future workers.[2] The University of Minnesota, particularly President COFFMAN, the editorial committee consisting of Dr. C. O. ROSENDAHL, Dr. GEORGE O. BURR, and Dr. ROSS AIKEN GORTNER, and all who participated in the project are to be complimented upon bringing this most worthy project to fruition.

The volume contains sufficient material for several other monographs or articles if the data were thoroughly analyzed, studied, and correlated. Part I consists of short papers and memoranda on the physico-chemical properties of plant saps in relation to plant geography. These are by Dr. HARRIS, with the exception of a short paper by Dr. H. L. SHANTZ on the importance of phytochemical studies in the field of plant geography.

Part II contains the experimental data systematically arranged by botanical names of species under each state. An easily understood code and symbols are used to facilitate publication in as brief form as possible. A separate index to the data has been appended for use by those wishing to make ecological studies of the plants in particular communities. This index will probably be used extensively in conjunction with the descriptions of stations.—C. F. KORSTIAN.

**Reproduction in fungi**

Following rather closely the appearance of the fifth volume, the sixth volume of BULLER's monumental work[3] is, in effect, a continuation of the second part of its predecessor, dealing as it does with reproductive mechanisms in various fungi, most of which involve violent discharge.

The present volume is divided into three parts, the first (and longest) being concerned with the genus *Pilobolus*. There is the usual complete survey of earlier literature, followed by a detailed account of the development of the *Pilobolus* sporangium and its discharge. Numerous illustrations show the manner in which the subsporangial swelling is acted upon by the incident rays of light so as to aim and discharge the sporangium in the direction of the light source. *Ocellus*, the term first suggested by BULLER in 1921 for the subspo-

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[2] HARRIS, J. ARTHUR, The physico-chemical properties of plant saps in relation to phytogeography. Completed and edited by C. O. ROSENDAHL, G. O. BURR, and R. A. GORTNER. Univ. Minnesota Press. pp. vi+339. 1934.

[3] BULLER, A. H. R., Researches on fungi. Vol. VI. pp. xii+513. figs. 231. Longmans, Green and Co., London. 1934.

rangial swelling, is chosen with evident intention to emphasize the eye-like character of the structure.

Taxonomists will welcome the systematic treatment of *Pilobolus* and *Pilaira*, contributed by W. B. GROVE, which constitutes the fourth chapter. On p. 190 the Pilobolidae is referred to as a "section of the Mucorini," while on p. 200 it is said to be a "family of the Mucoraceae." Presumably the author regards the group as a family, but if so, it is to be regretted that he has not seen fit to follow accepted modern usage and write Pilobolaceae.

The second part deals with the phenomenon of puffing in the Discomycetes. Working with representatives of several genera, and on the basis of experiment as well as observation, the author concludes that the simultaneous discharge of a large number of asci is of definite advantage to the fungus, in that it produces a blast of air capable of carrying the spores for several inches after they have lost their original velocity. The direction of discharge is governed in part by the position of the operculum and in part by heliotropic response of the ascus tip, the details varying in different species, but the aim always so adjusted as to secure a parallel direction of discharge for all asci in a single ascocarp, whatever their position in the hymenium.

The third part deals with pseudorhizae and gemmifers as organs of Hymenomycetes. BULLER adopts FAYOD'S term "pseudorhiza" as more accurate than the term "rooting base" used to designate the subterranean part of the stem of the basidiocarp of a number of fungi, and perhaps best illustrated in the familiar *Collybia radicata*. Study of the development of the pseudorhiza in connection with the subaerial stipe in *Coprinus macrorhizus* makes it clear that the pseudorhiza is merely the lower portion of the stipe, serving as a connecting link between a mycelium immersed in a buried matrix and the subaerial part of the fructification. Of particular interest is the perennial, branching pseudorhiza of *Collybia fusipes*.

The final chapter has to do with the so-called "Stilbum" heads of the coffee leaf-spot fungus, *Omphalia flavida*. It is shown that these are in reality sterile, modified basidiocarps, in which the entire pileus is detached and serves as a disseminule. Observations on the luminosity of the species are recorded and comparison is made with the reproductive bodies of *Sclerotium coffeicola*. STAHEL'S view that these may represent sterile, modified basidiocarps of a *Typhula* (essentially similar in nature to the gemmifers of the *Omphalia*) is quoted with approval.

As in the previous volumes, the numerous well selected and fully explained figures and the excellent general summary and index greatly facilitate the use of the volume.—G. W. MARTIN.

**A new mycological text**

There has long been need for a comprehensive treatment of the fungi in English, suitable for use with classes whose members have had no introduction

to the subject other than that received in elementary courses. Teaching mycologists will welcome, therefore, the appearance of BESSEY'S new book.[4]

After an introductory chapter, which includes a short history of mycology, a chapter is devoted to forms commonly included in the fungi, but which the author regards either as unrelated or at most doubtfully related to the true members of the group. Such forms include the slime molds, Acrasiales, Labyrinthulales, Plasmodiophorales, and Chytridiales. The true fungi are held to begin with the filamentous Phycomycetes, whose derivation from green algae is favored, while the higher fungi are regarded as constituting a distinct phylum, the Carpomycetae, traceable to the red algae. Quite properly, these views on phylogeny determine the order of presentation.

The book is well balanced. The Phycomycetes are adequately covered in three chapters (67 pages), with frequent reference to FITZPATRICK'S work, which, however, is by no means slavishly followed. The Carpomycetae are divided into three classes, the Ascomycetae, Teliosporae, and Basidiomycetae. The feminine plural, instead of the more familiar masculine ending, is adopted for all groups but the Fungi Imperfecti, presumably to emphasize the author's frequent allusion to the fungi as plants. The treatment of the Ascomycetes (127 pages) begins with the Laboulbeniales, Lecanorales, and Pezizales as showing most resemblance to the postulated red algal ancestral forms, and ends with the Aspergillales and Saccharomycetales, this sequence being adopted in accordance with a concept of progressive reduction. The introduction of the Lecanorales and Pyrenulales into the series is a welcome approach toward a more rational handling of the lichens.

The recognition of the Teliosporae, including the rusts and smuts, as a class coordinate with the other two groups of higher fungi (43 pages) is unlikely to find general acceptance. In accordance with this view, however, the terms basidium, basidiospore, and pycnium are regarded as inapplicable, the older terms promycelium, sporidium, and spermogonium being preferred. The pycniospores are called sperms. The author prefers to derive the Teliosporae from some primitive member of the Pezizales rather than directly from the red algae.

The Basidiomycetes, as restricted, occupy three chapters (60 pages). The Heterobasidiae are recognized in the page headings, but not the Homobasidiae. The treatment is on the whole conservative and in accord with established usage, with adequate recognition of its tentative character. A brief discussion of the imperfect fungi concludes the text.

To each chapter is appended an admirably selected bibliography, while a guide to the literature for identification of the fungi occupies no less than 76 pages at the end. The method of citing references by groups entails a certain

[4] BESSEY, E. A., A textbook of mycology. pp. xv+495. P. Blakiston's Son & Co., Philadelphia. 1935. $4.

amount of repetition, but this is probably justified by the resulting convenience and accessibility of the titles.

Typographical errors are not unduly numerous, but the addition of commas in a number of appropriate places would clarify the meaning and occasionally save the sentence, as printed, from approaching absurdity. The author properly argues that smut spores are not chlamydospores (p. 252) but permits himself (p. 95) to refer to the "sterigmata" of *Bremia*. The indiscriminate use of the terms "sexual" for any reproductive process involving or implying nuclear change, and "asexual" for those not involving such change, has been adequately criticized by LINK. It is to be regretted that the latter's proposed substitutes, caryallagic and acaryallagic, have not yet found their way into general use. A somewhat analogous criticism might be made of the frequent and often meaningless adjective "normal." The illustrations are abundant and for the most part helpful, although many of the drawings appear unnecessarily crude, and a few of the photographs, figs. 39 and 107 for example, would convey little to one who was not already familiar with the fungi they illustrate.

A satisfactory textbook in a specialized field, if it is to be of wide usefulness, must necessarily take a conservative position with reference to many debatable points, at the same time making it clear that there are points of view to be considered other than those adopted. This book conforms to such a criterion. The author's judicial attitude, his manifest fairness, the fullness with which he cites opposing views, and his constant suggestion of challenging problems combine to make this a useful and stimulating text.—G. W. MARTIN.

### Plant life

The list of elementary texts continues to grow. The present work[5] is appreciably shorter and less encyclopedic than some, being expressly designed for a course extending through a single semester.

On the whole, the book is well illustrated; some of the illustrations are refreshingly new and apt while many others are drawn from various popular texts and other sources. Although the author has deliberately centered his method of presentation around processes, structure is not neglected. In many instances, treatment of some of the subjects is abbreviated to little more than a mention of their existence or suspected importance. The usual sequence of forms purporting to represent evolution in plants is given.

One is still left to hope that eventually an introduction to the science of botany may be written and offered as a textbook in the subject. Perhaps the two things are mutually antagonistic. The hope may indeed be vain so long as instructors continue to demand longer and longer abbreviated encyclopedias for use as texts, and the struggle to keep up with this demand grows more and more acute.—E. J. KRAUS.

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[5] SWINGLE, D. B., Plant life. 8vo. pp. 441. figs. 290. Van Nostrand Co., New York. 1935. $3.

### Hortus

Those who have been accustomed to turn to Hortus for concise and accurate information on plants of horticultural importance will welcome this new edition,[6] which is brought up to date by the addition of a supplement. Reference to nearly 3000 additional species has been appended, as well as many varieties and form names. The same general style of citation as in previous editions has been followed.—E. J. KRAUS.

### Weeds

Botanists and agriculturists alike will welcome a comprehensive treatise on weeds.[7] The book is divided into two principal parts. Part I affords a history and much general ecological information on weeds, including methods of control. Part II is a systematic listing of a great number of species, together with specific notations of their occurrence and methods of control. An extensive key is provided. Good and clearcut line drawings are used to illustrate many of the species.—E. J. KRAUS.

### General plant cytology

The first volume of the second edition of TISCHLER'S work on plant cytology has recently appeared.[8] This volume deals exclusively with the resting nucleus, which is considered under the following headings: resting nucleus and its external morphology; chemical organization; morphological structure of the reticulum and the karyolymph; the nucleolus; protein crystals; relation of the nucleus to plastids, chondriosomes, centrosomes, and blepharoplast; relation of the nucleus to cell wall formation; movement of the nucleus within the cell and its physiological significance; relation of the nucleus to cell division; multinucleate condition of cells; and degeneration and resorption of the resting nuclei. The book is an able and comprehensive summary of the knowledge pertaining to this subject which has been accumulated during the past 75 years and should be a valuable addition to the reference library. The citation of pertinent literature is very complete.—J. M. BEAL.

### A Swiss flora

Two European ecologists have undertaken the task of an elaborate and detailed flora[9] of southeastern Switzerland. This mountainous region possesses an

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[6] BAILEY, L. H. and ETHEL Z., Hortus: a concise dictionary of gardening, general horticulture and cultivated plants in North America. New revised edition with supplement. pp. 755. pls. 16; figs. 22. Macmillan Co., New York. 1935. $5.

[7] MUENSCHER, W. C., Weeds. 8vo. pp. 577. figs. 123. Macmillan Co., New York. 1935. $6.

[8] TISCHLER, G., Allgemeine Pflanzenkaryologie. Handbuch der Pflanzenanatomie. Band. II. pp. 630. figs. 252. Verlag Borntraeger, Berlin. 1934. RM 64.

[9] BRAUN-BLANQUET, J., and RÜBEL, EDUARD, Flora von Graubünden. Veröffen. Geobot. Inst. Rübel in Zürich 7: Erste Lieferung. pp. 1–382. map. 1932; Zweite Lieferung. pp. 385–820. 1933; Dritte Lieferung. pp. 821–1204. 1934. Hans Huber, Bern and Berlin.

annual rainfall ranging from 90 to 150 cm. in the north to 140 to 160 cm. in the south. For the purpose of the flora the montane and lowland portions of the area are divided into a northern, a central, and a southern region dominated respectively by forests of beech, pine, and oak. The subalpine and alpine portions of the area, with similar major regions, are subdivided according to the various mountain systems. Special attention is given to the ecological range and habitat conditions of the various species and citations of altitude are many and detailed.

The nomenclature and arrangement are those of SCHINZ and KELLER in their Flora der Schweiz but numerous other authorities have been consulted, as indicated by the extensive bibliography. Many Swiss botanists have assisted in the collection and compilation of the data and many herbaria have been consulted.

The work will set a high standard for systematic botanists and will prove of great value to plant geographers and ecologists interested in the distribution and history of European floras. Students of alpine vegetation will find in it an abundance of ecological data from the home of alpine ecologists.

Three numbers have been issued. The first deals with the pteridophytes, gymnosperms, and monocotyledons; the second includes the lower families of the dicotyledons up to and including the Rosaceae; while the third discusses the intermediate families of the dicotyledons from the Leguminosae to the Solanaceae. It is expected that the concluding number of the volume will appear very soon.—G. D. FULLER.

### Struggle for existence

This volume[10] summarizes the opinions and theories of the most prominent contributors to our knowledge of this subject. It also reports a series of experimental tests of the mathematical theories of VOLTERRA, LOTKA, and BAILEY, made under rather carefully controlled laboratory conditions. From these experiments GAUSE is able to criticize constructively the present mathematical descriptions of data and to suggest scores of possible problems. His experimental data are concerned first with the struggle for a common limited amount of energy by various organisms, as for example yeast cells (*Saccharomyces cerevisiae vs. Schizsaccharomyces kephir*), and second with prey-predator relations between various species of *Paramecia* and *Didinium*.

The genuinely critical attitude of the author is stimulating, and the mathematical treatments are so simply explained that the book should aid the nonmathematically inclined experimenter to understand the highly technical works of LOTKA, VOLTERRA, and BAILEY.

The tone of the book is distinctly introductory and tentative, treating only a very small portion of the possible fields of investigation, and it is to be hoped that it will be followed by a more complete and comprehensive treatise on the subject.—R. B. OESTING.

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[10] GAUSE, G. F., The struggle for existence. pp. 163. Williams and Wilkins Co., 1934. $3.

### Southern California botany

Students and traveling botanists will welcome the appearance of this authoritative and compact treatment[11] of the plants of an area smaller in size than those covered by the larger works of ABRAMS and JEPSON. MUNZ acknowledges the influence of these authors' publications on his own volume, but points out that most of our western states are so poorly known botanically that any student of a local flora can quickly discover new facts. His observations over a period of nearly two decades are summarized in the present volume.

The area covered includes San Diego, Imperial, Los Angeles, Orange, Riverside, San Bernardino, and Ventura counties, portions of Santa Barbara, Kern, and Inyo counties, as well as the Channel Islands. It is thus a region of very diverse floristic, climatic, and topographic relationships. A hasty tabulation by the reviewer shows that approximately 2700 species and 750 genera are found in the area, of which a large number of the former are endemic. At one climatic extreme arctic-alpine elements are found on San Gorgonio and San Jacinto peaks, while at the other Lower Sonoran elements dominate in the Colorado and Mohave deserts.

A number of useful innovations not commonly found in manuals are a discussion of the distribution of southern California plants, a list of nomenclatorial changes, one of persons for whom species have been named, and a list of meanings of specific names, this last prepared by F. W. PEIRSON. The glossary and index seem adequate and accurate. The list of abbreviations of authors' names is very brief.

The descriptive flora covers 596 pages, the arrangement of families following the Englerian system in the main. Descriptions are adequate for the purposes of the book, and the keys seem to be usable. Occasional errors have crept in. The most serious one which caught the eye of the reviewer is in the generic key of the Ranunculaceae. Other errors are more apparently typographical in nature. Three of the author's students prepared the 310 text figures which are included.

Nomenclatorial changes number 98. The majority are new combinations, but two new species, *Phacelia minutiflora* J. Voss and *Monardella robisonii* Epling, as well as eleven new varieties, are described.

Preparation and publication of the book was made possible largely through the generosity of Miss ELLEN SCRIPPS.—C. E. OLMSTED.

### Fossil cycads

A recent paper[12] describes the well known *Cycad* trunk from the Carpathians of Poland which is now found in the Zwinger Museum at Dresden. The description includes the histological features of the woody cylinder, the cortical

[11] MUNZ, P. A., A manual of southern California botany. 8vo. pp. xl+642. figs. 310. Claremont, California. 1935.

[12] WIELAND, G. R., Fossil cycads, with special reference to *Raumeria reichenbachiana* Goeppert sp. of the Zwinger of Dresden. Palaeontographica. **79**: ser. B. 85–130. Stuttgart. 1934.

parenchyma, and the flowers. It is suggested that the generic name be changed to *Cycadeoidea*. WIELAND makes comparisons with other Cycadeoideae and establishes the great similarity of *Raumeria reichenbachiana* with *Cycadeoidea dakotensis*.—A. C. NOÉ.

### Low temperature relations of plants

For many years research concerning the low temperature relations of plants has been very active. The problems of freezing injury, the conditions which produce hardiness, the metabolic significance of the hardening processes, varietal differences in resistance to frost injury, inheritance of frost resistance, the significance of unfreezable water, etc., have received wide attention. Correspondingly an extensive literature has developed, widely scattered in journals, many of them obscure, or not obtainable readily in libraries.

An annotated bibliography covering this field of investigation has been prepared by HARVEY[13] and his assistants. The bibliography lists 3412 contributions, arranged alphabetically. A topical index of 28 pages makes it possible to secure the citations to almost any subject one desires. Wherever the titles do not indicate the subject matter of the papers, brief annotations give an idea of their contents.

The bibliography is mimeoprinted, with flexible leatherette binding. It represents a very large expenditure of time and energy. There is need of such bibliographies in the case of extensive and far flung literatures, and future progress will be much more rapid as a result of the unselfish devotion of the author and his aids, who have worked on the compilation for over 15 years. Their efforts will be appreciated by all workers in this field.—C. A. SHULL.

### Biochemical laboratory methods

MORROW's volume,[14] first published in 1927 shortly after the author's untimely death, has appeared in a second edition, revised and rewritten by W. M. SANDSTROM.[15] The principal changes made concern the order of the experiments, which have now been arranged to conform more closely to the order of presentation in the companion text, Outlines of Biochemistry, by R. A. GORTNER. It has been possible to omit some discussion which has been more adequately presented in GORTNER's book. The volume is therefore somewhat reduced in size, 319 instead of 350 pages. It offers 231 laboratory exercises of varying degrees of difficulty. The work has proved very useful, and the new edition will no doubt stimulate much interest in biochemical methods and problems.—C. A. SHULL.

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[13] HARVEY, R. B., An annotated bibliography of the low temperature relations of plants. 8vo. pp. ii+223. Burgess Publishing Co., Minneapolis. 1935. $4.

[14] BOT. GAZ. **85**:466–467. 1928.

[15] MORROW, C. A., and SANDSTROM, W. M., Biochemical laboratory methods for students of the biological sciences. 8vo. pp. xv+319. John Wiley and Sons, New York. 1935. $3.75.

# GENERAL INDEX

Classified entries will be found under Contributors and Reviews. New names and names of new genera and species are printed in **bold-face** type.


## A

Aborting fruits of Prunus 505
Abramson, H. A., "Electrokinetic phenomena and their application to biology and medicine" 380
Acorus calamus, seed and seedling 758
After-ripening treatment and germination 372
Alternation of generations in Ectocarpus siliculosus 421
Anatomy, of aerial roots of Vitis 367; of apple and peach roots 581; of vegetative organs of parsnip 44
Apple roots, temperature, growth, anatomy, and metabolism 581
Aquatic plants, chemical composition 186
Arber, Agnes, "The Gramineae: A study of cereal, bamboo, and grass" 383
Arnold, C. A. 180
Arthur, J. C., "Manual of the rusts in United States and Canada" 190
Ascomycetes, systematics 192
Avery, G. S. Jr. 314

## B

Bailey, L. H. and Ethel Z., "Hortus: a concise dictionary of gardening, general horticulture and cultivated plants in North America" 771
Baker, F. S., "Theory and practice of silviculture" 576
Baldwin, H. I. 372
Baldwin, J. T. Jr. 558
Bannan, M. W. 260
Basidiomycetae, spore germination 282
Beal, J. M. 196, 771
Beetles, pines infested with 709
Benedict, H. M. 330
Berry, E. W. 751
Bessey, E. A., "A textbook of mycology" 768
Bidens **collina** 144; **hivoana** 143; **kivuensis** 145; **onisciformis** 144
Biochemical laboratory methods 774
Biswell, H. H. 676
Blakeslee, A. F. 521
Botanical Review 577
Braun-Blanquet, J., "Flora von Graubünden" 771
Brodie, H. J. 533
Brown, C. A. 640
Brown, H. P., "Identification of the commercial timbers of the United States" 384
Bruce, D., "Forest mensuration" 580
Buell, M. F. 758
Buller, A. H. R., "Researches on fungi" 377, 767

## C

Cactaceae, chromosome numbers 565
Caird, R. W. 709
Calcium and ultraviolet radiation 330
California, southern, botany 773
Callixylon **whiteanum** 180
Cambial growth of certain conifers 73
Carotenoids, 378; methods for purification 447
Carya, embryogeny 93
Chamberlain, C. J. 191, 579
Chemical composition of aquatic plants 186
Chimaeras, plant 193
Chromosome numbers, of Cactaceae 565; numbers in Sedum 558
Chromosomes, of the soy bean 189; of Tradescantia 353
Cinnamomeae, Linneons in the American 228

Color of pollen in Petunia 342
Compositae, taxonomy 136
Conifers, cambial growth of certain 73; phylogeny 195
Contributors: Arnold, C. A. 180; Avery, G. S. Jr. 314; Baldwin, H. I. 372; Baldwin, J. T. Jr. 558; Bannan, M. W. 260; Beal, J. M. 196, 771; Benedict, H. M. 330; Berry, E. W. 751; Biswell, H. H. 676; Blakeslee, A. F. 521; Brodie, H. J. 533; Brown, C. A. 640; Buell, M. F. 758; Caird, R. W. 709; Chamberlain, C. J. 191, 579; Daniel, H. A. 186; Daugherty, L. H. 363; Edwards, T. I. 118; Erlanson, Eileen W. 197; Feng, C. F. 485; Ferguson, Margaret C. 342; Fuller, G. D. 195, 386, 387, 576, 577, 578, 771; Gould, Sophia A. 118; Hamly, D. H. 755; Harper, H. J. 186; Harrold, T. J. 505; Hayward, H. E. 383, 384, 385; Hougen, V. H. 389; Humphrey, L. M. 353; Hunt, Barbara 342; Imai, Y. 571; Kaufmann, F. H. O. 282; Kienholz, Raymond 73; Korstian, C. F. 767; Kraus, E. J. 770, 771; Kribs, D. A. 547; Langdon, LaDema M. 93; Lindstrom, E. W. 766; Link, G. K. K. 190, 192; Link, K. P. 468; Martin, G. W. 377, 767, 768; Miller, E. S. 447; Miyake, K. 571; Nightingale, G. T. 581; Noé, A. C. 194, 195, 196, 388, 773; Oesting, R. B. 772; O'Hanlon, Sister Mary Ellen 154; Olmsted, C. E. 381, 387, 580, 773; Papenfuss, G. F. 421; Pearl, Raymond 118; Ramaley, Francis 165, 579; Rees-Leonard, Olive L. 734; Satina, Sophia 521; Sax, Karl 353; Sherff, E. E. 136; Shull, C. A. 175, 193, 378, 380, 388, 575, 576, 774; Smith, A. H. 533; Stockwell, P. 565; Turner, L. M. 367; Veatch, C. 189; Voss, John 3; Voth, P. D. 298, 578; Walker, J. C. 468; Warning, Winifred C. 44; Weaver, J. E. 389; Weldon, M. D. 389
Coreopsis **curtisii** 146
Cosmos **herzogii** 148; **mattfeldii** 148
Cotton, genetical and cytological study of species hybrids 485
Cowles, Henry Chandler, retirement as editor 1
Crane, M. B., "The genetics of garden plants" 766
Cucumis seedlings, influence of temperature and nutrition on growth and life duration 118
Cycads, fossil 773
Cytological effects of a gene in Datura 521; study of Ectocarpus 421; study of species hybrids of cotton 485
Cytology, general plant 771

## D

Daniel, H. A. 186
Datura, cytological effects of a gene 521
Daugherty, L. H. 363
Dicotyledons, salient lines of structural specialization in wood rays 547
Dumortiera, comparative morphology 154
Dyad formation in sporogenesis 521

## E

Ecology of forest trees 676
Economic plants 385
Ectocarpus siliculosus, alternation of generations 421
Edwards, T. I. 118
Electrokinetic phenomena 380
Embryogeny of Carya and Juglans 93
Ephedra, vegetative phases 298
Erlanson, Eileen W. 197
Excretion in higher plants 575

## F

Feng, C. F. 485
Ferguson, Margaret C. 342
Ferns of the northwest 579
Fisher, R. A., "Statistical methods for research workers" 576
Flammula, transferred to Pholiota 533
Flora of Buffalo and vicinity 387
Florin, R., work of 194
Forest mensuration 580
Fossil, Cycadophytes and Cycadofilicales 194; pollen deposits and postglacial migration of forests 3; wood from Arizona 363
Frey-Wyssling, A., "Die Stoffausscheidung der höheren Pflanzen" 575
Frye, T. C., "Ferns of the northwest" 579
Fuller, G. D. 195, 386, 387, 576, 577, 578, 771
Fungi, reproduction 767; researches 377

## G

Garratt, G. A., "Systematic anatomy of the woods of the Myristicaceae" 196

Gause, G. F., "The struggle for existence" 772
Genetical study of species hybrids of cotton 485
Genetics of garden plants 766
Germination, of Basidiomycetae spores 282; of white pine 372
Gould, Sophia A. 118
Gramineae 383
Grout, A. J., "Moss flora of North America north of Mexico" 578
Gymnospermen, Embryologie der 191
Gymnosperms, origin and cellular character of xylem rays 260

## H

Haemolytic systems 388
Hagerup, O., "Zur Organogenie und Phylogenie der Koniferen-Zapfen" 195
Hamly, D. H. 755
Harper, H. J. 186
Harris, J. A., "The physico-chemical properties of plant saps in relation to phytogeography" 766
Harrold, T. J. 505
Harvey, R. B., "An annotated bibliography of the low temperature relations of plants" 774
Hawley, R. C., "The practice of silviculture with particular reference to its application in the United States of America" 576
Hayward, H. E. 383, 384, 385
Holman, R. M., "A textbook of general botany" 196
Hortus 771
Hougen, V. H. 389
House, H. D., "Wild flowers" 580
Hryniewiecki, B., "Précis de l'histoire de la botanique en Pologne" 578
Humphrey, L. M. 353
Hunt, Barbara 342
Hutchinson, J., "The families of flowering plants. Vol. II. Monocotyledons" 381

## I

Illumination and growth 579
Imai, Y. 571
Infestation of pines 709

## J

Juglans, embryogeny 93

## K

Kaufmann, F. H. O. 282
Kienholz, Raymond 73
Korstian, C. F. 767
Kraus, E. J. 770, 771
Krenke, N. P., "Wundkompensation, Transplantation und Chimären bei Pflanzen" 193
Kribs, D. A. 547

## L

Labiatae, taxonomy 136
Langdon, LaDema M. 93
Laurelia **guinazui** 752
Lawrence, W. J. C., "The genetics of garden plants" 766
Light, influence of supplemental on blooming 165; line in Melilotus alba 755
Lindstrom, E. W. 766
Link, G. K. K. 190, 192
Link, K. P. 468
Linneons in the American Cinnamomeae 228

## M

MacBride, T. H., "The Myxomycetes" 192
Macrogametophyte of Solanum tuberosum 734
Martin, G. W. 377, 767, 768; "The Myxomycetes" 192
Melilotus alba, light line in 755
Methods, for the purification of the common carotenoids 447; statistical, for research workers 576
Migration of forests, postglacial 3
Miller, E. S. 447
Miyake, K. 571
Molisch, Hans, "Erinnerungen und Welteindrücke eines Naturforschers" 388
Monimiaceae and a new Laurelia 751
Monocotyledons, families of 381
Morrow, C. A., "Biochemical laboratory methods for students of the biological sciences" 774
Moss flora of North America 578

Muenscher, W. C., "Weeds" 771
Munz, P. A., "A manual of southern California botany" 773
Mycological text, new 768
Myrrh 196
Myxomycetes 192

N

Nannfeldt, J. A., "Studien über die Morphologie und Systematik der Nichtlichenisierten Inoperculaten Discomyceten" 192
Nightingale, G. T. 581
Noé, A. C. 194, 195, 196, 338, 773
Nomenclature, committee on, "Glossary of terms used in describing woods" 195
Nutrition and growth of Cucumis seedlings 118

O

Odontia, morphology and biology 640
Oesting, R. B. 772
O'Hanlon, Sister Mary Ellen 154
Olmsted, C. E. 381, 387, 580, 773
Onion bulb parasites and toxicity of phenolic compounds 468

P

Panchaud, J., "Action du milieu extérieur sur le métabolisme végétal" 579
Panshin, A. J., "Identification of the commercial timbers of the United States" 384
Papenfuss, G. F. 421
Parsnip, anatomy of vegetative organs 44
Peach roots, temperature and growth 581
Pearl, Raymond 118
Persistence of subspecific types of Xanthium under field conditions 175
Petunia, pollen color 342
Pharbitis nil, maternal transmission of mutated plastids 571
Phenols, toxic 468
Pholiota (Flammula) polychroa 533
Phosphorus and ultraviolet radiation 330
Phyllostegia **bracteata** 137; **helleri** 138; **lantanoides** 137; **rockii** 139; **wawrana** 139
Physico-chemical properties of plant saps 766
Pines infested with bark beetles 709
Pinus, seed germination 372
Poland, botany in 578
Pollen, deposits and postglacial migration of forests 3; of Petunia 342
Ponder, Eric, "The mammalian red cell and the properties of haemolytic systems" 388
Postglacial migration of forests and fossil pollen deposits 3
Prunus, aborting fruits 505
Purification of the common carotenoids, methods for 447

R

Radiation, ultraviolet, and calcium and phosphorus contents 330
Ramaley, F. 165, 579
Raunkiaer, C., "The life forms of plants and statistical plant geography" 387
Rays in gymnosperms, origin and cellular character of xylem 260
Record, S. J., "Identification of the timbers of temperate North America" 386; "Rôle of wood anatomy in taxonomy" 196
Rees-Leonard, Olive L. 734
Reviews: Abramson's "Electrokinetic phenomena and their application to biology and medicine" 380; Arber's "The Gramineae: A study of cereal, bamboo, and grass" 383; Arthur's "Manual of the rusts in United States and Canada" 190; Bailey's "Hortus: a concise dictionary of gardening, general horticulture and cultivated plants in North America" 771; Baker's "Theory and practice of silviculture" 576; Bessey's "A textbook of mycology" 768; Braun-Blanquet's "Flora von Graubünden" 771; Brown's "Identification of the commercial timbers of the United States" 384; Bruce's "Forest mensuration" 580; Buller's "Researches on fungi" 377, 767; Crane's "The genetics of garden plants" 766; Fisher's "Statistical methods for research workers" 576; Frey-Wyssling's "Die Stoffausscheidung der höheren Pflanzen" 575; Frye's "Ferns of the northwest" 579; Garratt's "Systematic anatomy of the woods of the Myristicaceae" 196; Gause's "The struggle for existence" 772; Grout's "Moss flora of North America north of Mexico" 578; Ha-

gerup's "Zur Organogenie und Phylogenie der Koniferen-Zapfen" 195; Harris's "The physico-chemical properties of plant saps in relation to phytogeography" 766; Harvey's "An annotated bibliography of the low temperature relations of plants" 774; Hawley's "The practice of silviculture with particular reference to its application in the United States of America" 576; Holman's "A textbook of general botany" 196; House's "Wild flowers" 580; Hryniewiecki's "Précis de l'histoire de la botanique en Pologne" 578; Hutchinson's "The families of flowering plants. Vol. II. Monocotyledons" 381; Krenke's "Wundkompensation, Transplantation und Chimären bei Pflanzen" 193; Lawrence's "The genetics of garden plants" 766; MacBride's "The Myxomycetes" 192; Martin's "The Myxomycetes" 192; Molisch's "Erinnerungen und Welteindrücke eines Naturforschers" 388; Morrow's "Biochemical laboratory methods for students of the biological sciences" 774; Muenscher's "Weeds" 771; Munz's "A manual of southern California botany" 773; Nannfeldt's "Studien über die Morphologie und Systematik der Nichtlichenisierten Inoperculaten Discomyceten" 192; Panchaud's "Action du milieu extérieur sur le métabolisme végétal" 579; Panshin's "Identification of the commercial timbers of the United States" 384; Ponder's "The mammalian red cell and the properties of haemolytic systems" 388; Raunkiaer's "The life forms of plants and statistical plant geography" 387; Record's "Identification of the timbers of temperate North America" 386; Record's "Rôle of wood anatomy in taxonomy" 196; Robbins' "A textbook of general botany" 196; Rübel's "Flora von Graubünden" 771; Sandstrom's "Biochemical laboratory methods for students of the biological sciences" 774; Schnarf's "Embryologie der Gymnospermen" 191; Schumacher's "Forest mensuration" 580; Stanford's "Economic plants" 385; Steuer's "Myrrhe und Stakte" 196; Stout's "Day lilies" 577; Swingle's "A textbook of systematic botany" 195; Swingle's "Plant life" 770; Tischler's "Allgemeine Pflanzenkaryologie. Handbuch der Pflanzenanatomie" 771; Wieland's "Fossil cycads, with special reference to Raumeria reichenbachiana Goeppert sp. of the Zwinger of Dresden" 773; Zechmeister's "Carotenoide" 378; Zenkert's "The flora of the Niagara frontier region" 387

Revision of Rosa sp. 197

Robbins, W. W., "A textbook of general botany" 196

Root distribution, relation to organic matter in prairie soil 389; habits of certain deciduous forest trees 676

Roots, temperature and growth of apple and peach 581

Rosa, species revision 197

Rübel, Eduard, "Flora von Graubünden" 771

Rusts 190

## S

Sandstrom, W. M., "Biochemical laboratory methods for students of the biological sciences" 774

Satina, Sophia 521

Sax, Karl 353

**Schildera adamanica,** a new fossil wood 363

Schnarf, Karl, "Embryologie der Gymnospermen" 191

Schumacher, F. X., "Forest mensuration" 580

Sedum, somatic chromosome numbers 558

Sherff, E. E. 136

Shull, C. A. 175, 193, 378, 380, 388, 575, 576, 774

Silviculture 576

Smith, A. H. 533

Soil, effects on root habit 676; root distribution and organic matter in prairie 389

Solanum tuberosum, macrogametophyte 734

Soy bean, chromosomes 189

Spores of Basidiomycetae, germination 282

Stanford, E. E., "Economic plants" 385

Stenogyne **calycosa** 142; **scandens** 143; **sororia** 142

Steuer, R. O., "Myrrhe und Stakte" 196

Stockwell, P. 565

Stout, A. B., "Day lilies" 577

Swingle, D. B., "A textbook of systematic botany" 195; "Plant life" 770

Swiss flora 771
Systematic botany, textbook 195
Systematics of the Ascomycetes 192

T

Taxonomic descriptions, usefulness of wood in 195
Temperature and growth of apple and peach roots 581; and growth of Cucumis seedlings 118; low, relations of plants 774
Timbers, identification of commercial 384
Tischler, G., "Allgemeine Pflanzenkaryologie. Handbuch der Pflanzenanatomie" 771
Tobacco plants, structural responses to the practice of topping" 314
Topping tobacco plants 314
Toxicity of phenolic compounds to onion bulb parasites 468
Tradescantia, chromosomes 353
Turner, L. M. 367

U

Ultraviolet radiation and calcium and phosphorus contents 330

V

Veatch, C. 189
Vitis rotundifolia, anatomy of aerial roots 367
Voss, John 3
Voth, P. D. 298, 578

W

Walker, J. C. 468
Warning, Winifred C. 44
Weaver, J. E. 389
Weeds 771
Weldon, M. D. 389
Wieland, G. R., "Fossil cycads, with special reference to Raumeria reichenbachiana Goeppert sp. of the Zwinger of Dresden" 773
Wild flowers 580
Wood identification 386; rays of dicotyledons 547
Wound compensation 193

X

Xanthium, persistence of subspecific types under field conditions 175

Z

Zechmeister, L., "Carotenoide" 378
Zenkert, C. A., "The flora of the Niagara frontier region" 387